# An Exploratory Analysis of Regional Assessment Data in Support of Nutrient Criteria Development for EPA Regions 5, 7, and 8 

Prepared for:
United States Environmental Protection Agency, Region 5
Chicago, IL

## Prepared by:

Ted R. Angradi
United States Environmental Protection Agency
Office of Research and Development
National Health and Environmental Effects Research Laboratory
Mid-Continent Ecology Division
Duluth, MN
March 14, 2011

## Executive summary

EPA recognizes the importance of nutrient criteria in protecting designated uses from eutrophication effects associated with phosphorus and nitrogen and has worked with states over the past 12 years to assist them in developing nutrient criteria. Towards that end, EPA has provided states and tribes with technical guidance to assess nutrient impairment and develop ecoregion-specific criteria. EPA published eco-regional criteria recommendations in 2000-2001 based on a frequency distribution approach meant to approximate reference condition concentrations. EPA also published recommendations in 2000 on scientifically defensible empirical approaches for setting numeric criteria. In November 2010, EPA elaborated on one of these empirical approaches in its publication, "Using Stressor-response Relationships to Derive Numeric Nutrient Criteria."

In developing nutrient criteria based on stressor-response analyses, States generally have relied on data from only within their boundaries. Using data from only within a state's boundaries works well where a significant portion of the state is relatively un-disturbed. In some locations, such as the central part of the United States, significant human-caused nutrient-related disturbance is widespread, and analysis of nutrient-related data within a state may not always show a consistent, strong relationship between nutrient enrichment and biological condition. In locations of significant nutrient-related disturbance, other parameters compete with nutrients in terms of impacting biology or otherwise confounding the identification of nutrient-biology relationships. High sediment loads can lead to light limitation, making the streams unresponsive to nutrients. Intermittent toxicity to plants and algae due to agricultural chemicals could also mask expected responses in Illinois streams. Nutrient concentrations may be above levels at which biological response would normally occur; subsequently, analysis may not observe much response as nutrient concentrations change. Where such conditions exist across most of a state, strong biological response to nutrients may not consistently be observed if using data from only within a state.

The purpose of this project was to identify nutrient response relationships from a cross-regional data set that could be used by individual states across the data analysis area. The analysis considers nutrient and biotic assemblage (macroinvertebrates) data across the Plains, Corn Belt, and Upper Midwest Regions from EPA's Wadeable Streams Assessment Survey. The analysis results herein suggest that data for large parts of EPA Regions 5, 7, and 8 could be aggregated for purposes of nutrient response threshold analysis, based on similarities in expected biological community.

This report should be considered as an exploratory effort in identifying types of analyses and stressor-response relationships that could be pursued in subsequent analyses. Given that the analysis results in this report tend to be consistent with the growing body of research on nutrient response thresholds, the report results could also be used as a line(s) of evidence for states and tribes in developing nutrient criteria.

## Disclaimer

The analyses and opinions expressed in this report are the author's and do not reflect the policy of the U.S. Environmental Protection Agency, the Office of Research and Development, or the Mid-Continent Ecology Division.

## Background

In April 2010, EPA staff at the Mid-Continent Ecology Division in Duluth, MN were asked by EPA Region 5 to analyze existing datasets to determine if they might be useful for setting nutrient criteria for Region 5 states (Ohio, Illinois, Indiana, Michigan, Wisconsin, and Minnesota). At that time, the only available dataset was from the 2004 Wadeable Streams Assessment (WSA, USEPA 2006). Analysis of additional datasets may be undertaken as they become available.

## Purpose

The purpose of this report was to address the following questions:

1) Determine if there is a contiguous region of the central United States across which biological expectations are similar. Specifically,
a. Determine whether the sampled macroinvertebrate assemblages are dissimilar among WSA aggregated or level 3 ecoregions.
b. Determine whether the assemblages in the three level 3 ecoregions that constitute the Corn Belt Plains are dissimilar from assemblages elsewhere in the Midwest.
2) Conduct exploratory analyses across the central United States to identify candidate nutrient threshold values (CTVs) based on multiple analysis methods that could be used by states and tribes as lines of evidence in nutrient criteria development.
3) Determine how the thresholds from the analyses compare to published thresholds.
4) Based on the exploratory analyses, identify nutrient response relationships and types of analyses that merit additional evaluation.

## Data and analyses

The data used in this analysis were extracted from the WSA dataset (http://www.epa.gov/owow/streamsurvey/web_data.html). Files containing stream water chemistry data, site information data, benthos metrics, benthos counts (genus level), and physical habitat data were downloaded for all WSA sites. National Land Cover Database (NLCD) 2001 data were compiled for all sites in the Plains and Upper Midwest (Fig. 1). Plains and Upper Midwest are defined for WSA as shown in Fig. 2. The Plains and Upper Midwest includes the following aggregate ecoregions: Upper Midwest, Temperate Plains, Southern Plains, and Northern Plains). Level 3 ecoregions in this report are from Omernik (1987). Land cover data does not include watershed area in Canada. Records without total N or total P data were deleted. Preliminary analysis identified one site with only three macroinvertebrate taxa in the sample, which was deleted. Site revisits were excluded from this analysis. The final dataset had WSA data for 1371 sites with NLCD data for 327 Plains/Upper Midwest sites (Fig. 1). Analyses were
conducted using SAS System for Windows v.9.1 statistical software, Sigmaplot for Windows v. 11 graphics and statistical software, and PRIMER v. 6 software for multivariate analysis. Information on WSA sample design and field methods are available online:
http://water.epa.gov/type/rsl/monitoring/streamsurvey/index.cfm
The analysis in this report includes classification of Plains and Upper Midwest WSA sites (objective 1) and derivation of candidate threshold values (objectives 2-3). Nine different methods (Table 1) were used to derive candidate threshold values (CTV). Graphical, regression and multivariate methods were used to examine the data. The following analyses were performed:

Analysis 1:
ANOSIM (Primer), a multivariate ANOVA analog, was used to test if sites from different groups had similar assemblages using the $R$-statistic. When $R$ is large ( $\gg 0.2$ ) the assemblages are relatively dissimilar (i.e., there is a strong group effect); when $R$ is small ( $\ll 0.2$ ) there is high overlap in assemblages between groups. ANOSIM was used to compare WSA aggregated ecoregions (Fig. 2), level 3 ecoregions (if $n \geq 5$ sites), Plains/Upper Midwest sites in the Corn Belt plains and not in the Corn Belt plains, and between groups of sites based on natural variation (northern and southern streams, steep and flat streams, streams with large and small watersheds, and streams with fine and coarse dominant substrate). Based on the ANOSIM results, sites in the Plains/Upper Midwest aggregated ecoregions (Upper Midwest, Southern Plains, Northern Plains, Temperate Plains) were retained for further analyses ( $n=327$ ).

## Analysis 2:

Means and selected percentiles of nutrient concentrations were computed for all Plains/Upper Midwest sites, for WSA aggregated ecoregions, and for sites in the Corn Belt Plans (CBP) and sites not in then CBP, reference and non-reference sites, and sites in Illinois or Indiana and sites not in Illinois or Indiana (CTV methods 1 and 2, Table 1). Reference sites were identified in the WSA data site. For an explanation of the WSA reference approach, go to http://water.epa.gov/type/rsl/monitoring/streamsurvey/upload/2007 5_9_streamsurvey 04_chap 1-5-2-07.pdf.

Analysis 3:
Principal components analysis (PCA, PRIMER) was used to generate a human disturbance gradient (PC 1) based on the percent of the site's watershed in row crops, pasture or hay, development, forest, or wetlands, and riparian disturbance and predict "background" nutrient concentrations. Human population density, road density, and canopy openness were not included in the PCA because of missing values in the source data. The PCA is an alternative approach to the multiple linear regression approach described below (Analysis 4) that allows the inclusion of natural land covers (forest, wetland) in the gradient. Simple and piecewise three-segment linear regression (Sigmaplot) was used to predict the "background" nutrient concentration ( $y$ ) at a site with the lowest value for PC 1 (CTV method 4, Table 1).

Analysis 4:
Multiple linear regression (SAS) was used to predict the "background" nutrient concentration ( $y$ ) at sites with all human disturbances $(x)$ set to background concentration (i.e., the $y$-intercept)
(CTV method 3, Table 1). Human disturbance variables included the percent of the site's watershed in row crops, pasture or hay, and development, the human population of the watershed, the road density, the riparian condition, and the percent riparian canopy openness ( 100 - percent canopy coverage). Mallow's $C p$ statistic was used to select the best models without over-fitting (http://www.statistics4u.info/fundstat_eng/cc_varsel_mallowscp.html).

## Analysis 5:

Multiple linear regression was used to predict macroinvertebrate metric values at all sites using natural variation variables (latitude, longitude, mean stream wetted width, mean channel slope, dominant substrate particle size, watershed area, and annual precipitation). Regression residuals, representing variation in metrics not explained by natural variables, were retained for further analysis.

## Analysis 6:

Piecewise linear regression (Sigmaplot) was used to determine "breakpoints" in relationships between twenty-four non-redundant ( $r_{\mathrm{s}}<0.85$ ) "responsive" macroinvertebrate assemblage metrics ( $y$ ) and log-transformed nutrient concentrations ( $x$ ). Responsive metrics were those that were at least weakly rank correlated ( $r_{\mathrm{s}} \geq 0.25$ ) with at least one nutrient. Other methods for breakpoint (or change point) analysis are available, but the piecewise approach is straightforward, relatively easy to implement, and the results are amenable to comparison with hypothesized responses.

The choice of a three-segment regression was based on a hypothetical three-segment response to increasing nutrient concentration (Fig. 3A and 3B). In this idealized response, metrics values are all high (plot A) or all low (for metrics that increase with nutrients, plot B) until a "response threshold" (Rt) is reached. Metric values then decrease (or increase) to a secondary threshold (St) beyond which they do not change much. The midpoint of this response is at $R m$, which is less protective than $R t$ because some decrease in the metric has already occurred. $R m$ represents the middle of the range over which the metric responds most strongly to nutrients. St (as in Fig. 3A and 3B) is undesirable as a threshold since it may mark the beginning of an alternative stable state in the macroinvertebrate fauna from which ecological recovery may be difficult (Dodds et al 2010). The dashed lines in Fig 3 indicate that there can be some deviation from a perfectly stable metric in the sub-threshold range.

In an alternative three-segment response (Fig 3C and 3D), metrics values decline from the minimum observed nutrient concentration, $M n$, to a secondary threshold at St. The biological interpretation of this response is not clear since there is no response threshold. The midpoint of this response at $R m$ is not necessarily protective because the true value of $R t$ may occur below the observed range of nutrient concentrations.

If there were no significant breakpoints in the three-segment model, a two-segment piecewise regression was fit to the data. The interpretation of the hypothesized two-segment response (Fig. 3 E and 3 F ) is similar to the three-segment response (Fig 3A and 3B) except that there is no secondary threshold (St becomes $M x$, or the maximum observed value). The interpretation of the
response depicted in Fig. 3G and 3H is analogous to the three-segment response depicted in Fig. 3 C and 3D.

If there were no significant breakpoints in the two-segment model, a simple linear regression was fit to the data.

The interpretation of the biological meaning of breakpoints is somewhat subjective. Where appropriate, the first breakpoint with increasing nutrient concentration ( $R t$ ) was defined as the candidate threshold value (CTV). $R t$ is a protective threshold that conforms to the hypothesized response. In some cases, $R m$ was selected even when the response resembled the hypothesized response (Figs. 3A, 3B, 3E, or 3F) because of high variability or sparse data when Rt occurred at the extreme of the measured nutrient data. In other cases, the only significant breakpoint was detected at such an extreme value that the relationship was considered linear and no threshold was inferred.

A breakpoint in a metric response to a nutrient is not necessarily biologically relevant or appropriate for threshold determination. Breakpoint analysis can be potentially misleading especially if applied systematically without an underlying biological model. In this report, every breakpoint was individually examined with respect to the hypothesized responses and the variability and density of data near the breakpoint. The rationale for each CTV based on a breakpoint is documented in an appendix to this report.

The piecewise regression analysis was conducted on raw metric values (CTV method 5, Table 1 ), on the regression residuals described above (CTV method 6, Table 1), and on raw metric values for each of two stream groups: streams with coarse substrate ( $\geq 1 \mathrm{~mm}$ ), and streams with fine substrate ( $<1 \mathrm{~mm}$ ) (CTV method 7, Table 1).

CTV values derived from biotic responses to nutrient concentrations were plotted against the $r^{2}$ value of the associated simple linear or breakpoint regression to determine if CTV converges on a threshold value as predictive power increases.

## Analysis 7:

Interpolation of simple linear regressions of raw biotic data ( $y$ ) with nutrient concentrations ( $x$ ) were used to derive reference nutrient concentration for each biotic metric (CTV method 8 , Table 1). Reference expectations (the value for the biotic metric at reference sites) were based on a percentile of the reference population as defined for WSA. The same analysis was separately conducted for streams with fine and coarse substrate. (CTV method 9, Table 1). CTV values derived from biotic responses to nutrient concentrations were plotted against the $r^{2}$ value of the associated simple linear or breakpoint regression to determine if CTV converges on a threshold value as predictive power increases.

## Analysis 8:

From percentage frequency distributions, the percent of Plains/Upper Midwest streams and the percent of streams in Indiana and Illinois with nutrient concentrations above a CTV were determined.

Analysis 9:
CTVs derived from the preceding analyses were compared to published threshold values for the Plains and Upper Midwest.

## Findings

## Spatial variation in macroinvertebrate assemblages (Analysis 1)

Many comparisons between WSA aggregated ecoregions were significant (Table 2). However, none of the comparisons with a strong group effect included comparisons between WSA aggregated ecoregions in the Plains and Upper Midwest. Only one comparison (Upper Midwest compared to the Southern Plains) had a moderate groups effect ( $R=0.30$ ). The other comparisons between Plains/Upper Midwest aggregated ecoregions were weak or negligible. Assemblages in streams in the Corn Belt Plains were similar to assemblages in non-Corn Belt Plains streams. Assemblages in streams in Illinois and Indiana were similar to assemblages in other states.

Many comparisons among assemblages for level 3 Plains ecoregions were significant (39\%, Table 3). The strongest distinctions (highest $R$ statistics) were generally for widely separated ecoregions. Only three significant comparisons were for ecoregions that were contiguous (i.e., shared a border). The large number of significant comparisons between relatively small regions across a vast area is not surprising, but the results must be interpreted with caution because of small sample sizes.

Nutrient concentrations (Analysis 2)
Mean total N concentration was highest in the Temperate Plains ( $5252 \mathrm{ug} / \mathrm{L}$ ) and lowest ( $<1350$ $\mathrm{ug} / \mathrm{L}$ ) in the Northern and Southern Plains (Table 4). Mean total P concentration was highest in the Northern and Temperate Plains aggregated ecoregions ( $\geq 240 \mathrm{ug} / \mathrm{L}$ ) and lowest in the UMW aggregated ecoregion ( $85 \mathrm{ug} / \mathrm{L}$ ).

Mean total N concentration was higher in the Corn Belt Plains ( $8539 \mathrm{ug} / \mathrm{L}$ ) than elsewhere in the Plains/Upper Midwest (1674 ug/L). Mean total P concentration was similar in both groups (212 and $234 \mathrm{ug} / \mathrm{L}$, Table 4).

Sites identified as reference in the WSA dataset had much lower mean total N and total P concentrations than other non-reference sites (Table 4). Sites in Illinois and Indiana had a higher mean total N concentration than other plains sites. Mean total P concentration was the same at sites in Illinois and Indiana as sites elsewhere in the Plains/Upper Midwest.

For level 3 ecoregions with $\geq 8$ sites, total N concentration was highest in the Central and Western Corn Belt Plains (>9600 ug/L, Table 5), and lowest in the Northern Lakes and Forests and North Central Hardwoods ( $<600 \mathrm{ug} / \mathrm{L}$ ). Total P concentration (when $n \geq 8$ ) was highest in the Northwestern Glaciated Plains and Central Great Plains (>430 ug/L) and was lowest in Northern Lakes and Forests (42 ug/L).

## Predicting "background nutrient" concentration from PCA (Analysis 3)

Human disturbance varied among aggregated WSA ecoregions (Table 6). Percent of the watershed in row crops was highest in the Temperate Plains ( $60 \%$ ) and lowest in the Northern and Southern Plains ( $18 \%$ ). Watersheds in WSA aggregated ecoregions were 5-7 percent developed except in the Northern Plains, which was less developed (2\%). Percent forest and wetland were much higher in the Upper Midwest ( $57 \%$ combined) than other WSA aggregated ecoregions ( $\leq 20 \%$ combined). Population density and road density followed a similar pattern. Riparian disturbance and canopy openness were lower at Upper Midwest sites than at sites elsewhere in the Plains/Upper Midwest.

Human disturbance was consistently greater in the Corn Belt Plains and non-reference sites than at non Corn Belt Plains and reference sites (Table 6).

The first principal component explained $35 \%$ of the variation in the ordination and was primarily a gradient from relatively undisturbed Upper Midwest sites with a high percentage of forest and wetlands in their watershed to sites (including virtually all Temperate Plains sites) with a relatively high percentage of row crops, development, and riparian disturbance (Fig. 4). The second principal component ( $24 \%$ of variance explained) separated Northern and Southern Plains sites with high riparian disturbance but low percentage of forest, wetland, pasture/hay, and development, from the other WSA aggregated ecoregions.

## Predicting background nutrient concentration from human disturbance (Analysis 4)

A multiple regression model that included percent row crops, percent pasture/hay, road density, and canopy openness predicted a background total N concentration at a Plains/Upper Midwest site of $306 \mathrm{ug} / \mathrm{L}\left(r^{2}=0.54\right.$, Table 7). A weaker model $\left(r^{2}=0.17\right)$ that included percent row crops, percent pasture/hay, population density, riparian disturbance, and canopy openness predicted a background total $P$ concentration of $26 \mathrm{ug} / \mathrm{L}$.

Predicted background total N concentration (Table 7) was highest at Northern Plains sites (526 ug/L) and lowest at Temperate Plains sites ( $288 \mathrm{ug} / \mathrm{L}$ ). Background total P concentration could only be reliably predicted for the Southern Plains ( $23 \mathrm{ug} / \mathrm{L}$ ) and Upper Midwest ( $19 \mathrm{ug} / \mathrm{L}$ ).

Predicted background total N concentration at Corn Belt Plains sites was $333 \mathrm{ug} / \mathrm{L}$, but could not be reliably predicted for total P. Predicted background total N and total P concentration for a site in Illinois or Indiana was 196 and $34 \mathrm{ug} / \mathrm{L}$, respectively (Table 7).

A three-segment piecewise regression model explained $46 \%$ of the variation in total N concentration with PC 1 (Fig. 5A). This regression approach was used because there seemed to be different relationships between human disturbance (PC1) and total N for each of three parts of the disturbance range (the range of mostly Upper Midwest sites, the range of mostly Northern + Southern Plains sites, and the range of mostly Temperate Plains sites). At sites with minimal disturbance (lowest value for PC 1), which were Upper Midwest sites with a high percentage of forest and wetland (Fig 5), the predicted background total N concentration was $436 \mathrm{ug} / \mathrm{L}$. A
linear regression model explained $19 \%$ of the variation in total P concentration with PC 1 . The predicted background total P concentration was $16 \mathrm{ug} / \mathrm{L}$ at sites with minimum human disturbance (Fig. 5B).

## Macroinvertebrate metric relationship to natural variation (Analysis 5)

All natural factors had a significant effect on at least some macroinvertebrate metrics (Appendix 1). Substrate had the highest (based on $F$-statistics) and most consistent significant effect on metric values, followed by latitude. Mean values for natural variables are given in Appendix 2.

Substrate was significantly rank correlated with longitude, latitude, mean slope, mean stream width, and precipitation (Appendix 3). Of the natural factors operating at a site, macroinvertebrate assemblages are likely to be most influenced by substrate characteristics.

Streams were divided into two substrate group for further analysis: streams with fine substrate (< 1 mm mean diameter) and streams with coarse substrate ( $\geq 1 \mathrm{~mm}$ mean diameter) (Appendix 4). Plots of macroinvertebrate metrics with substrate size (Fig. 6) show that the biologically significant substrate size cut-off probably varies among metrics (e.g., > 1 mm for HBI and TL89PTAX; < 1 mm for TL03PIND and EPT_RICH), but 1 mm (log-transformed value of 0 mm ) is a reasonable approximation of the critical value.

Reference sites had a greater percentage of sites with coarse substrate (62\%) than fine substrate (38\%). For all other groups, more sites had fine substrate. Overall, 70 percent of sites had fine substrate. Mean diameter of substrate in streams in each substrate group are given in Appendix 3.

Results of the multiple regressions based on individual metrics (Appendix 1) are not corroborated by ANOSIM results (Table 2). Across all sites, assemblages in streams with fine substrates were not dissimilar to assemblages in streams with coarse substrates ( $R=0.03$ ). Dissimilarity between WSA aggregated ecoregions was greater than variation between natural groups, suggesting that at a regional scale, biogeographic variation (e.g., in species pool composition) accounts for more variation than local factors.

## Candidate threshold values based on macroinvertebrate metric response to nutrients breakpoint regression (Analysis 6)

Using breakpoint regression, a candidate threshold value (CTV) could be derived for the response of most metrics to nutrient concentration (Tables 8-11). Exceptions were when there was no significant breakpoint (the relationship was linear) or when breakpoints occurred near an extreme of the data and were judged unreliable (how the CTV was determined for each metric is documented in Appendix 5; data plotted in Appendix 6).

For the response of raw metrics to total N concentration, there was one extreme value (12,022 $\mathrm{ug} / \mathrm{L}$ ) for OLLEPTAX, which is based on a very tolerant group, the Oligochaeta (See Appendix 10 for explanation of metric names). Median CTV for all metrics was $379 \mathrm{ug} / \mathrm{L}$ total N (from Table 8).

The same analysis conducted using the regression residuals (from models summarized in Table 8) instead of the raw metrics produced generally similar results. Although a few more metrics lacked or had unreliable breakpoints, the median CTV ( $388 \mathrm{ug} / \mathrm{L}$ ) was similar to the median CTV for raw metrics ( $379 \mathrm{ug} / \mathrm{L}$ ). Median strength of models based on $r^{2}$ was similar for raw metrics ( 0.10 ) and regression residuals ( 0.11 ).
Plotting CTV for total N against the $r^{2}$ value for the piecewise regression (Fig. 7A) shows that as $r^{2}$ increases, the CTV converges on a value between 100 and $1000 \mathrm{ug} / \mathrm{L}$ for both the raw metrics and the CTVs based on the regression residuals.

For the response of raw metrics to total P, there were two extreme CTVs, 371 and $561 \mathrm{ug} / \mathrm{L}$, for HPRIME and HBI, respectively. HBI is a positive metric that increases with increasing pollution and the high CTV may reflect the response of the most tolerant organisms in the sample. Median CTV for all metrics was $17 \mathrm{ug} / \mathrm{L}$ total P (Table 9).

The same analysis conducted using the regression residuals (Table 9) instead of the raw metrics produced generally similar results. The median CTV ( $20 \mathrm{ug} / \mathrm{L}$ total P ) was similar to the median CTV for raw metrics ( $17 \mathrm{ug} / \mathrm{L}$ ). Median strength of models based on $r^{2}$ was slightly higher for raw metrics (0.11) than for models based on regression residuals (0.07).

Plotting CTV for total P against the $r^{2}$ value for the piecewise regression (Fig. 7B) shows that as model fit increases, variation in the CTVs decreases for both the raw metrics and the models based on the regression residuals. CTV values associated with higher $r^{2}$ values were generally within a range of $10-100 \mathrm{ug} / \mathrm{L}$ total P .

Separate analysis for streams with fine and coarse substrate had only a small effect on the results for total N (Table 10). Median $r^{2}$ and CTV values for total N in streams with fine substrate were about the same as for all streams combined. (Table 8 and 10; data plotted in Appendix 7). Median $r^{2}$ and CTV values for total N in streams with coarse substrate were also similar to all streams combined.

Classifying the streams by substrate size had a greater effect on results for total P (Table 9-11; data plotted in Appendix 5). Median $r^{2}$ for streams with fine substrates (0.08) were similar to all streams ( 0.11 ). However, median $r^{2}$ for streams with coarse substrates $(0.20)$ was higher than the median $r^{2}$ for all streams ( 0.07 ). The reason for the stronger relationships for streams with coarse substrate is not known, but is probably related to the degree of impairment these streams. Reference sites, most of which have coarse substrate (Appendix 4), are steeper (Appendix 2), and more agricultural (Table 6). There were probably more relative unimpaired sites among coarse substrate streams allowing for better detection of a response by macroinvertebrates to nutrients.

Plotting CTV for total N and total P against the $r^{2}$ value for the piecewise regression (Fig. 8) shows that as model fit increases, variation in the CTVs decreases for both streams with fine and coarse substrates.

## Candidate threshold values based on macroinvertebrate metric response to nutrients interpolation of linear regression (Analysis 7)

Using interpolation of simple linear regression, a candidate threshold value (CTV) could be derived for the response of metrics to nutrient concentration (Tables 12-17). This method requires reliable reference expectations for each metric. For the WSA data, metric values for the worst reference sites (the $25^{\text {th }}$ or $75^{\text {th }}$ percentile of reference sites, depending on whether the metrics increased or decreased with increasing nutrients) were, in most cases, worse than metric values for the best non-reference sites (the $25^{\text {th }}$ or $75^{\text {th }}$ percentile of non-reference sites, depending on whether the metrics increased or decreased with increasing nutrients). Because of this, the reference expectation corresponding to the median metric value for the reference sites was use in the interpolations.

The CTVs for total N based on interpolation for all streams ranged from 2 to $5570 \mathrm{ug} / \mathrm{L}$, with a median CTV or $347 \mathrm{ug} / \mathrm{L}$ (Table 12). Because of the weak relationships between nutrients and metrics ( $r^{2} \leq 0.15$ ), the prediction intervals were very wide (Table 12, Appendix 8 ).

The CTVs for total P based on interpolation for all streams ranged from 2 to $436 \mathrm{ug} / \mathrm{L}$, with a median CTV of $21 \mathrm{ug} / \mathrm{L}$ (Table 13). Because of the weak relationships between nutrients and metrics ( $r^{2} \leq 0.20$ ), the prediction intervals were very wide (Table 13, Appendix 8).

Plotting CTVs against the $r^{2}$ value for the interpolations (Fig. 9) shows that as model fit increases, variation in the CTVs decreased. CTV values associated with higher $r^{2}$ values were generally with a range of $100-1000 \mathrm{ug} / \mathrm{L}$ total N and $10-100 \mathrm{ug} / \mathrm{L}$ total P .

The CTVs for total N based on interpolation for streams with fine substrate ranged from 0 to $7369 \mathrm{ug} / \mathrm{L}$, with a median CTV of $164 \mathrm{ug} / \mathrm{L}$ (Table 14, Appendix 9). The CTV values for total N based on interpolation for streams with coarse substrate ranged from 4 to $13701 \mathrm{ug} / \mathrm{L}$, with a median CTV of $636 \mathrm{ug} / \mathrm{L}$ (Table 15, Appendix 9).

The CTVs for total P based on interpolation for streams with fine substrate ranged from $<0$ to $1461 \mathrm{ug} / \mathrm{L}$, with a median CTV of $10 \mathrm{ug} / \mathrm{l}$ (Table 16). The CTV values for total P based on interpolation for streams with coarse substrate ranged from 6 to $294 \mathrm{ug} / \mathrm{L}$, with a median CTV of $43 \mathrm{ug} / \mathrm{L}$ (Table 17).

Plotting CTVs against the $r^{2}$ value for the substrate-specific interpolations (Fig. 10) shows that as model fit increases, variation in the CTVs decreased. CTV values associated with higher $r^{2}$ values were generally with a range of $100-1000 \mathrm{ug} / \mathrm{L}$ total N and $10-100 \mathrm{ug} / \mathrm{L}$ total P .

## Regional assessment based on derived CTVs (Analysis 8)

Cumulative percentage distribution curves (Fig. 11) show the percentage of stream sites that would have a nutrient concentration above a particular value. The curves are based on single water grab samples at each site, so the percentages must be considered provisional. Across the range of criteria derived from WSA data in this report (about 100-1000 ug/L total M, and 10-100 $\mathrm{ug} / \mathrm{L}$ total P ), the percent of Plains/Upper Midwest streams exceeding the total N criteria ranges from 53 to $99 \%$. The percent of streams in Indiana and Illinois exceeding the total N criteria
ranges from 72 to $100 \%$. The percent of Plains/Upper Midwest streams exceeding the total P criteria ranges from 45 to $95 \%$. The percent of streams in Indiana and Illinois exceeding the total N criteria ranges from 43 to $100 \%$.

## Comparisons with published criteria (Analysis 9)

The CTV range for total N and total P suggested by biotic response to nutrients (100 to 1000 $\mathrm{ug} / \mathrm{L}$ total N and $10-100 \mathrm{ug} / \mathrm{L}$ total P ) bounds the other CTVs derived from predictive models and percentiles of the population (Fig. 12). Most published threshold values fall in this same range. Exceptions include values reference-percentile based value of Herlihy and Sifneos (2008) which is based on reference sites in the Corn Belt Plains, and the population based percentile values of Robertson et al. 2001).

## Major conclusions

1) Compared to dissimilarity between macroinvertebrate assemblages in super-regions and between all pairs of aggregated WSA ecoregions, dissimilarity between Plains/Upper Midwest aggregate ecoregions was low. Assemblages in streams in the Corn Belt Plains were not different from streams elsewhere in the Plains/Upper Midwest.
2) Among aggregated WSA ecoregions, mean total N was by far the highest in the Temperate Plains ( $5252 \mathrm{ug} / \mathrm{L}$ ). Mean total P was highest in the Northern Plains ( $283 \mathrm{ug} / \mathrm{L}$ ) and nearly as high in the Temperate Plains ( $240 \mathrm{ug} / \mathrm{L}$ ).
3) Background nutrient concentration in a Plains/Upper Midwest wadeable stream predicted from linear regression of PC1 were $436 \mathrm{ug} / \mathrm{L}$ total N and $16 \mathrm{ug} / \mathrm{L}$ total P .
4) Background nutrient concentrations in a Plains/Upper Midwest wadeable stream predicted from multiple linear regression were $306 \mathrm{ug} / \mathrm{L}$ total N and $26 \mathrm{ug} / \mathrm{L}$ total P. Predicted background concentrations for Illinois and Indiana were lower for total N ( $196 \mathrm{ug} / \mathrm{l}$ ) and slightly higher for total P ( $34 \mathrm{ug} / \mathrm{L}$ ), but sample size was small, and these estimates lack precision. Percent row crops was a significant parameter in nearly all models.
5) Separate analysis of the macroinvertebrate response to nutrient concentration for streams with fine and coarse substrates resulted in stronger relationships for total $P$ in streams with coarse substrate. Substrate-specific analysis could be used to adjust regional or state criteria.
6) The range of CTVs herein based on macroinvertebrate metric responses to nutrient concentration generally overlapped potential regional nutrient criteria derived by other methods both in the literature and from this study.

The determination of thresholds based on breakpoints in this report was, in some cases, partly subjective because of variability at the extremes of the data range and/or weak responses to nutrients for some metrics. Despite the subjectivity in determining the CTVs for each metricnutrient combination, a weight of evidence-based threshold is possible because CTVs tend to
converge (variability decreases) as the strength of piecewise regression models increases ( $r^{2}$ increases).
7) If states adopted nutrient criteria for the Plains/ Upper Midwest set at the low end of the range suggested by the CTVs presented here ( $100 \mathrm{ug} / \mathrm{L}$ total N and $10 \mathrm{ug} / \mathrm{L}$ total P), most stream sites $(>95 \%)$ would exceed nutrient criteria. If states adopted nutrient criteria that were set at the upper end of the range suggested by the CTVs presented here ( $1000 \mathrm{ug} / \mathrm{L}$ total N and $100 \mathrm{ug} / \mathrm{L}$ total P), about half of the stream sites would exceed nutrient criteria.
8) In this exploratory analysis, most of the biotic responses to nutrient concentration were weak, with $r^{2}$ values mostly $<0.2$ which means that less than $20 \%$ of the variation in biotic metrics was explained by variation in nutrient concentration. It may be that most streams are already significantly impaired by nutrients beyond the range of conditions over which a response to nutrients can be detected. Natural and sampling variation also confounds biotic responses. Some of the variation can be attributed to data quality from the Wadeable Stream Assessment. Estimates of nutrient concentration are based on a single summer grab sample. Estimates of macroinvertebrate assemblage metrics are based on single summer composite sample. Summer base flow conditions may not reflect the strongest response of many macroinvertebrate assemblage metrics to nutrient stress.

## References

Dodds, W.K., and R.M. Oakes. 2004. A technique for establishing reference nutrient concentrations across watersheds affected by humans. Limnology and Oceanography: Methods 2:333-341

Dodds, W.K., W.H. Clements, K. Gido, R.H. Hilderbrand, and R.S. King. 2010. Thresholds, breakpoints, and nonlinearity in freshwaters as related to management. Journal of the North American Benthological Society 29:988-997.

Herlihy, A.T., and J.C. Sifneos. 2008. Developing nutrient criteria and classification schemes for wadeable streams in the conterminous US. Journal of the North American Benthological Society 27:932-948.

Miltner R.J. 2010. A method and rationale for deriving nutrient criteria for small rivers and streams in Ohio. Environmental Management 45:842-588.

Omernik, J.M. 1987. Ecoregions of the conterminous United States. Annals of the Association of American Geographers 77:118-125.

Robertson, D.M., D.A. Saad, and A.M. Wieben. 2001. An alternative regionalization scheme for defining nutrient criteria for rivers and streams. USGS Water-Resources Investigations Report 01-4073.

Robertson, D.M, B.M. Weigel, and D.J. Graczyk. 2008. Nutrient concentrations and their relations to the biotic integrity of nonwadeable rivers in Wisconsin. USGS Professional Paper 1754.

Smith, R.A., R.B. Alexander, and G.E. Schwarz. 2003. Natural background concentrations of nutrients in streams and rivers of the conterminous United States. Environmental Science and Technology 37:3039-3047.
USEPA. 2006. Wadeable streams assessment: A collaborative survey of the Nation's streams. United States Environmental Protection Agency, EPA/841/B-06/002.

Wang, L. D.M. Robertson, and P.J. Garrison. 2007. Linkages between nutrients and assemblages of macroinvertebrates and fish in wadeable streams: Implication to nutrient criteria development. Environmental Management 39:194-212.

Table 1. Summary of methods used in this report to derive candidate threshold values (CTV) for total N and total P .

|  | Method | Groups |
| :---: | :---: | :---: |
| 1 | Percentile of the population | Multiple |
| 2 | Percentile of reference population | Multiple |
| 3 | Multiple linear regression predicting CTV from stressor variables | WSA Plains/Upper Midwest |
| 4 | Simple linear regression predicting CTV from PCA stressor gradient | WSA Plains/Upper Midwest |
| 5 | Piecewise regression of raw biotic data with nutrient concentration. CTVs based on breakpoint | WSA Plains/Upper Midwest |
| 6 | Piecewise regression of residual values with nutrient concentration. CTVs based on breakpoint | WSA Plains/Upper Midwest |
| 7 | Piecewise regression of raw biotic data with nutrient concentration data split by substrate size. CTV based on breakpoint | WSA Plains/Upper Midwest |
| 8 | Interpolation of CTVs for reference condition from linear regression of raw biotic data with nutrient concentrations. | WSA Plains/Upper Midwest |
| 9 | Interpolation of CTVs for reference condition from linear regression of raw biotic data with nutrient concentrations data split by substrate size | WSA Plains/Upper Midwest |

Table 2. $R$-statistics for comparisons of macroinvertebrate assemblage composition (based on genera) between selected ecoregional and natural variation groups. High values of $R$ indicate dissimilar assemblages. Highlighted comparisons are WAS aggregated Plains/Upper Midwest ecoregions. WMT $=$ Western Mountains, CPL $=$ Coastal Plain, SPL $=$ Southern Plains, TPL $=$ Temperate Plains, SAP = Southern Appalachians, NAP = Northern Appalachians, XER = Xeric, UMW = Upper Midwest, NPL = Northern Plains.

| Groups | $R$ | Interpretation |
| :---: | :---: | :---: |
| CPL, WMT | 0.84 | Strong group effect |
| TPL, WMT | 0.75 | Strong group effect |
| SPL, WMT | 0.73 | Strong group effect |
| WMT, NPL | 0.72 | Strong group effect |
| SAP, WMT | 0.62 | Strong group effect |
| UMW, WMT | 0.55 | Strong group effect |
| SAP, SPL | 0.52 | Strong group effect |
| CPL, XER | 0.50 | Strong group effect |
| NAP, SPL | 0.47 | Strong group effect |
| SAP, NPL | 0.47 | Strong group effect |
| NAP, WMT | 0.44 | Moderate group effect |
| NAP, NPL | 0.44 | Moderate group effect |
| SAP, XER | 0.42 | Moderate group effect |
| NAP, CPL | 0.39 | Moderate group effect |
| TPL, XER | 0.38 | Moderate group effect |
| TPL, NAP | 0.37 | Moderate group effect |
| SAP, CPL | 0.34 | Moderate group effect |
| WMT, XER | 0.33 | Moderate group effect |
| TPL, SAP | 0.32 | Moderate group effect |
| UMW, SPL | 0.30 | Moderate group effect |
| CPL, NPL | 0.28 | Moderate group effect |
| XER, NPL | 0.27 | Moderate group effect |
| SPL, XER | 0.27 | Moderate group effect |
| UMW, CPL | 0.26 | Weak group effect |
| UMW, NPL | 0.26 | Weak group effect |
| CPL, SPL | 0.25 | Weak group effect |
| TPL, SPL | 0.23 | Weak group effect |
| UMW, SAP | 0.23 | Weak group effect |
| TPL, CPL | 0.20 | Weak group effect |
| TPL, UMW | 0.18 | Negligible group effect |
| UMW, NAP | 0.17 | Negligible group effect |
| NAP, XER | 0.17 | Negligible group effect |
| SPL, NPL | 0.16 | Negligible group effect |
| UMW, XER | 0.14 | Negligible group effect |
| TPL, NPL | 0.13 | Negligible group effect |
| NAP, SAP | 0.10 | Negligible group effect |
| Indiana or Illinois, not Indiana or Illinois (Plains/Upper Midwest) | <0.01 | Group effect not significant |
| Corn Belt Plains, not Corn Belt Plains (Plains/Upper Midwest) | $<0.01$ | Group effect not significant |
| Aggregated WSA ecoregions (overall effect) | 0.19 | Weak group effect |
| Small watersheds ( $<100 \mathrm{~km}^{2}$ ), large watersheds ( $\left.>100 \mathrm{~km}^{2}\right)^{1}$ | 0.07 | Group effect not significant |


| Steep streams $(\geq 1 \%)$, flat streams $(<1 \%)^{2}$ | 0.03 | Group effect not significant |
| :--- | :--- | :--- |
| Fine substrate $(<1 \mathrm{~mm})$; coarse substrate $\geq 1 \mathrm{~mm})^{3}$ | 0.03 | Group effect not significant |
| Northern streams $(\geq 40 \mathrm{~N})$, southern streams $(<40 \mathrm{~N})^{4}$ | 0.07 | Group effect not significant |

${ }^{1}$ Group means ( $95 \%$ CI): small, $35(31-39) \mathrm{km}^{2}$, large, 2903 (1918-3887) $\mathrm{km}^{2}$
${ }^{2}$ Group means ( $95 \%$ CI): steep, 1.5 (1.4-1.6) \%, flat, 0.37 (0.33-0.40) \%
${ }^{3}$ Group means ( $95 \%$ CI): fines, $0.10(0.08-0.12) \mathrm{mm}$, coarse, $4.5(2.5-5.9) \mathrm{mm}$
${ }^{4}$ Group means ( $95 \%$ CI): north, 44.5 (44.2-44.8) dd, south, 37.4 (36.8-28.0) dd

Table 3. $R$ statistics for significant comparisons of macroinvertebrate assemblage composition between level 3 ecoregions (i.e., groups) with $\mathrm{n} \geq 5$. High values for $R$ indicate dissimilar assemblages. 60 of 153 pairwise comparisons were significant. Highlighted comparisons are level 3 ecoregions that were contiguous with each other. Sample sizes in Table 5.

| Groups | $R$ |
| :---: | :---: |
| DRIFTLESS AREA, CENTRAL OLKAHOMA/TEXAS PLAINS | 0.74 |
| S. MICHIGAN/N. INDIANA DRIFT PLAINS, WESTERN HIGH PLAINS | 0.71 |
| DRIFTLESS AREA, NORTHERN GLACIATED PLAINS | 0.64 |
| SOUTHEASTERN WISCONSIN TILL PLAINS, S. MICHIGAN/N. INDIANA DRIFT PLAINS | 0.64 |
| DRIFTLESS AREA, CENTRAL GREAT PLAINS | 0.64 |
| INTERIOR RIVER VALLEYS AND HILLS, WESTERN HIGH PLAINS | 0.63 |
| DRIFTLESS AREA, INTERIOR RIVER VALLEYS AND HILLS | 0.63 |
| DRIFTLESS AREA, LAKE AGASSIZ PLAIN | 0.62 |
| NORTH CENTRAL HARDWOOD FORESTS, NORTHERN GLACIATED PLAINS | 0.61 |
| DRIFTLESS AREA, CENTRAL IRREGULAR PLAINS | 0.60 |
| CENTRAL OLKAHOMA/TEXAS PLAINS, SOUTHEASTERN WISCONSIN TILL PLAINS | 0.59 |
| EASTERN CORN BELT PLAINS, WESTERN HIGH PLAINS | 0.59 |
| NORTH CENTRAL HARDWOOD FORESTS, CENTRAL OLKAHOMA/TEXAS PLAINS | 0.57 |
| WESTERN HIGH PLAINS, NORTHERN GLACIATED PLAINS | 0.57 |
| INTERIOR RIVER VALLEYS AND HILLS, NORTH CENTRAL HARDWOOD FORESTS | 0.55 |
| DRIFTLESS AREA, EASTERN CORN BELT PLAINS | 0.54 |
| NORTHERN LAKES AND FORESTS, SOUTHWESTERN TABLELANDS | 0.52 |
| NORTH CENTRAL HARDWOOD FORESTS, LAKE AGASSIZ PLAIN | 0.51 |
| DRIFTLESS AREA, S. MICHIGAN/N. INDIANA DRIFT PLAINS | 0.50 |
| NORTHERN LAKES AND FORESTS, WESTERN HIGH PLAINS | 0.50 |
| WESTERN HIGH PLAINS, LAKE AGASSIZ PLAIN | 0.45 |
| NORTHERN LAKES AND FORESTS, CENTRAL GREAT PLAINS | 0.45 |
| S. MICHIGAN/N. INDIANA DRIFT PLAINS, NORTHERN GLACIATED PLAINS | 0.43 |
| CENTRAL IRREGULAR PLAINS, NORTH CENTRAL HARDWOOD FORESTS | 0.43 |
| SOUTHEASTERN WISCONSIN TILL PLAINS, NORTHERN GLACIATED PLAINS | 0.42 |
| NORTHERN LAKES AND FORESTS, LAKE AGASSIZ PLAIN | 0.42 |
| NORTH CENTRAL HARDWOOD FORESTS, EASTERN CORN BELT PLAINS | 0.41 |
| CENTRAL GREAT PLAINS, S. MICHIGAN/N. INDIANA DRIFT PLAINS | 0.41 |
| INTERIOR RIVER VALLEYS AND HILLS, SOUTHWESTERN TABLELANDS | 0.39 |
| EASTERN CORN BELT PLAINS, NORTHERN GLACIATED PLAINS | 0.39 |
| DRIFTLESS AREA, NORTHERN LAKES AND FORESTS | 0.38 |
| S. MICHIGAN/N. INDIANA DRIFT PLAINS, LAKE AGASSIZ PLAIN | 0.38 |
| CENTRAL OLKAHOMA/TEXAS PLAINS, EASTERN CORN BELT PLAINS | 0.37 |
| WESTERN CORN BELT PLAINS, NORTHWESTERN GLACIATED PLAINS | 0.37 |
| INTERIOR RIVER VALLEYS AND HILLS, NORTHWESTERN GLACIATED PLAINS | 0.37 |
| WESTERN CORN BELT PLAINS, SOUTHWESTERN TABLELANDS | 0.37 |
| INTERIOR RIVER VALLEYS AND HILLS, S. MICHIGAN/N. INDIANA DRIFT PLAINS | 0.36 |
| NORTH CENTRAL HARDWOOD FORESTS, S. MICHIGAN/N. INDIANA DRIFT PLAINS | 0.36 |
| NORTHERN LAKES AND FORESTS, NORTHWESTERN GLACIATED PLAINS | 0.35 |
| CENTRAL OLKAHOMA/TEXAS PLAINS, NORTHERN GLACIATED PLAINS | 0.35 |
| WESTERN CORN BELT PLAINS, NORTHERN LAKES AND FORESTS | 0.34 |
| SOUTHWESTERN TABLELANDS, S. MICHIGAN/N. INDIANA DRIFT PLAINS | 0.34 |


| DRIFTLESS AREA, NORTHWESTERN GLACIATED PLAINS | 0.34 |
| :--- | :---: |
| WESTERN CORN BELT PLAINS, DRIFTLESS AREA | 0.34 |
| CENTRAL OLKAHOMA/TEXAS PLAINS, NORTHERN LAKES AND FORESTS | 0.34 |
| WESTERN CORN BELT PLAINS, CENTRAL OLKAHOMA/TEXAS PLAINS | 0.33 |
| CENTRAL IRREGULAR PLAINS, S. MICHIGAN/N. INDIANA DRIFT PLAINS | 0.33 |
| INTERIOR RIVER VALLEYS AND HILLS, LAKE AGASSIZ PLAIN | 0.32 |
| CENTRAL CORN BELT PLAINS, CENTRAL OLKAHOMA/TEXAS PLAINS | 0.32 |
| SOUTHWESTERN TABLELANDS, NORTHWESTERN GREAT PLAINS | 0.32 |
| CENTRAL GREAT PLAINS, SOUTHWESTERN TABLELANDS | 0.32 |
| NORTHERN LAKES AND FORESTS, NORTHERN GLACIATED PLAINS | 0.31 |
| CENTRAL OLKAHOMA/TEXAS PLAINS, LAKE AGASSIZ PLAIN | 0.30 |
| SOUTHWESTERN TABLELANDS, NORTHERN GLACIATED PLAINS | 0.27 |
| INTERIOR RIVER VALLEYS AND HILLS, NORTHERN GLACIATED PLAINS | 0.26 |
| CENTRAL IRREGULAR PLAINS, SOUTHWESTERN TABLELANDS | 0.26 |
| SOUTHWESTERN TABLELANDS, LAKE AGASSIZ PLAIN | 0.22 |
| CENTRAL IRREGULAR PLAINS, NORTHERN GLACIATED PLAINS | 0.20 |
| CENTRAL IRREGULAR PLAINS, LAKE AGASSIZ PLAIN | 0.18 |
| WESTERN CORN BELT PLAINS, NORTHWESTERN GREAT PLAINS | 0.17 |

Table 4. Mean, $95 \%$ confidence intervals, minimum, maximum, and candidate threshold values (CTV) based on percentiles for nutrient concentration in Plains/Upper Midwest streams, WSA aggregated plains ecoregions (see Table 1), and Corn Belt Plains (CBP) groups, reference and non-reference sites, and sites in Illinois or Indiana and sites not in Illinois or Indiana. Percentiles are $25^{\text {th }}$ (and $10^{\text {th }}$ for UMW) except "reference" for which percentiles are $75^{\text {th }}$. WSA aggregate ecoregions: SPL $=$ Southern Plains, TPL = Temperate Plains, UMW = Upper Midwest, NPL = Northern Plains. Other groups: $\mathrm{CBP}=$ Corn Belt Plains, $\mathrm{IL}=\mathrm{Illinois}, \mathrm{IN}=\mathrm{Indiana}$.

| Sites | $n$ | Total N (ug/L) |  |  |  |  | Total P (ug/L) |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | Mean | 95\%CI | Min | Max | CTV | Mean | 95\%CI | Min | Max | CTV |
| All | 327 | 2955 | 2411-3498 | 109 | 43650 | 616 | 216 | 163-269 | 2 | 5418 | 34 |
| TPL | 130 | 5252 | 4079-6551 | 238 | 43650 | 1046 | 240 | 164-317 | 15 | 4175 | 65 |
| UMW | 55 | 1778 | 1018-2536 | 160 | 15650 | 508 (311) | 85 | 51-120 | 3 | 618 | 17 (9) |
| NPL | 94 | 1345 | 839-1850 | 114 | 18775 | 569 | 283 | 139-426 | 2 | 5418 | 37 |
| SPL | 48 | 1236 | 808-1664 | 109 | 6981 | 414 | 169 | 74-265 | 3 | 2034 | 16 |
| CBP | 61 | 8539 | 6409-10669 | 376 | 43650 | 2292 | 234 | 91-378 | 15 | 4175 | 61 |
| Not CBP | 266 | 1674 | 1374-1974 | 109 | 18775 | 556 | 212 | 155-269 | 2 | 5418 | 29 |
| Reference | 50 | 840 | 574-1105 | 109 | 4454 | 900 | 48 | 34-62 | 2 | 181 | 69 |
| Not Reference | 277 | 3337 | 2707-3966 | 131 | 43650 | 3295 | 247 | 184-309 | 4 | 5418 | 232 |
| IN and IL | 28 | 5149 | 2593-7705 | 376 | 33350 | 889 | 216 | 86-347 | 29 | 1632 | 58 |
| Not IN or IL | 299 | 2749 | 2206-3293 | 109 | 43650 | 583 | 216 | 158-273 | 2 | 5418 | 32 |

Table 5. Mean nutrient concentration, percent of watershed area in row crops, and percent canopy openness by level 3 ecoregion.

| Level 3 ecoregion | $n$ | Mean <br> total N <br> $(\mathrm{ug} / L)$ |  | Mean <br> total P <br> $(\mathrm{ug} / \mathrm{L})$ | Row <br> crops <br> $(\%)$ |
| :--- | ---: | ---: | ---: | ---: | ---: |
| WESTERN CORN BELT PLAINS | 42 | 9652 | 274 | 79 | Canopy <br> openness <br> $(\%)$ |
| CENTRAL CORN BELT PLAINS | 10 | 9612 | 133 | 67 | 35 |
| SOUTHEASTERN WISCONSIN TILL PLAINS | 5 | 6706 | 54 | 56 | 26 |
| DRIFTLESS AREA | 11 | 5773 | 166 | 43 | 49 |
| HURON/ERIE LAKE PLAINS | 3 | 3803 | 157 | 63 | 19 |
| NORTHWESTERN GLACIATED PLAINS | 13 | 2530 | 452 | 21 | 23 |
| NORTHERN GLACIATED PLAINS | 16 | 2504 | 365 | 46 | 51 |
| EASTERN CORN BELT PLAINS | 9 | 2153 | 162 | 74 | 48 |
| INTERIOR RIVER VALLEYS AND HILLS | 17 | 2080 | 237 | 50 | 16 |
| CENTRAL GREAT PLAINS | 11 | 2004 | 438 | 50 | 27 |
| LAKE AGASSIZ PLAIN | 13 | 1805 | 307 | 66 | 38 |
| SOUTHWESTERN TABLELANDS | 18 | 1231 | 75 | 8 | 42 |
| CENTRAL IRREGULAR PLAINS | 13 | 1216 | 148 | 13 | 41 |
| TEXAS BLACKLAND PRAIRIES | 2 | 1181 | 163 | 2 | 16 |
| S. MICHIGAN/N. INDIANA DRIFT PLAINS | 9 | 1156 | 114 | 40 | 13 |
| NORTHWESTERN GREAT PLAINS | 81 | 1155 | 255 | 17 | 12 |
| FLINT HILLS | 2 | 1102 | 224 | 4 | 46 |
| NORTH CENTRAL HARDWOOD FORESTS | 8 | 958 | 89 | 24 | 18 |
| WESTERN HIGH PLAINS | 5 | 893 | 28 | 12 | 24 |
| CENTRAL OLKAHOMA/TEXAS PLAINS | 9 | 814 | 154 | 10 | 30 |
| NORTHERN MINNESOTA WETLANDS | 1 | 638 | 45 | 1 | 32 |
| NEBRASKA SAND HILLS | 1 | 613 | 86 | 1 | 19 |
| NORTHERN LAKES AND FORESTS | 26 | 599 | 42 | 5 | 78 |
| EDWARDS PLATEAU | 2 | 190 | 5 | 0 | 27 |

Table 6. Mean values for human disturbance variables. See Appendix 10 for explanation of variables. WSA aggregate ecoregions: $\mathrm{SPL}=$ Southern Plains, TPL = Temperate Plains, UMW = Upper Midwest, NPL = Northern Plains. Other groups: CBP = Corn Belt Plains, IL = Illinois, IN = Indiana.

| Sites ( $n$ ) | Row crops (\%) | Pasture/Hay (\%) | Developed (\%) | Forest (\%) | Wetlands (\%) | Population density (no./km ${ }^{2}$ ) | Road density (km/km ${ }^{2}$ ) | Riparian Disturbance (Index score) | Riparian canopy openness (\%) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| All (327) | 34.8 | 8.8 | 5.5 | 15.9 | 3.4 | 19.4 | 1.4 | 1.3 | 35.5 |
| TPL (130) | 59.6 | 13.1 | 7.5 | 8.5 | 1.9 | 25.2 | 1.8 | 1.5 | 32.3 |
| UMW (55) | 20.8 | 10.1 | 5.3 | 44.8 | 12.1 | 21.8 | 1.3 | 0.5 | 22.5 |
| NPL (94) | 17.5 | 5.4 | 2.1 | 7.5 | 1.6 | 1.5 | 0.9 | 1.4 | 46.5 |
| SPL (48) | 17.6 | 2.0 | 6.6 | 19.4 | 0.9 | 33.4 | 1.4 | 1.4 | 37.5 |
| CBP (61) | 76.5 | 4.6 | 9.2 | 4.6 | 0.6 | 35.5 | 2.2 | 1.6 | 30.7 |
| Not CBP (266) | 25.2 | 9.7 | 4.6 | 18.5 | 4.0 | 15.6 | 1.2 | 1.2 | 36.6 |
| Reference (50) | 20.3 | 9.0 | 3.5 | 30.8 | 3.4 | 6.5 | 1.1 | 0.9 | 27.0 |
| Not Reference (277) | 37.4 | 8.7 | 5.8 | 13.2 | 3.4 | 21.9 | 1.5 | 1.3 | 37.0 |
| IN and IL (28) | 66.6 | 6.3 | 13.1 | 13.0 | 0.1 | 70.6 | 1.9 | 1.7 | 24.3 |
| Not IN or IL (299) | 31.8 | 9.0 | 4.7 | 16.2 | 3.7 | 14.4 | 1.3 | 1.2 | 36.5 |

Table 7. Significant linear regression models for predicting nutrient concentrations (CTVs) at background human disturbance (backtransformed $y$-intercept). Disturbance data are from NLCD 2001 (\% land cover), and WSA site data. $D f$ is degrees of freedom; $C p$ is Mallows statistic which was used to select the most adequate significant model; prediction is the back transformed $y$-intercept (the nutrient concentration in ug/L at a site with no disturbance). WSA aggregate ecoregions: SPL = Southern Plains, TPL=Temperate Plains, UMW = Upper Midwest, NPL = Northern Plains. Other groups: CBP = Corn Belt Plains, IL = Illinois, IN = Indiana.

| Sites | Y <br> (logtransform ed) | $y$-intercept (SE) | Regression coefficients |  |  |  |  |  |  | Error Df | $r^{2}$ | $C p$ | $\begin{gathered} \text { CTV } \\ (\mathrm{ug} / \mathrm{L}) \end{gathered}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | Row crops \% | Pasture/ hay \% | Develop ment \% | Population density No/km ${ }^{2}$ | Road density Km/km ${ }^{2}$ | Riparian disturbance Score | $\begin{gathered} \hline \text { Canopy } \\ \text { openness } \\ \% \end{gathered}$ |  |  |  |  |
| All | Total N | 2.49 (0.05) | 0.0104 | 0.0038 |  |  | 0.1152 |  | 0.0022 | 307 | 0.54 | 5.34 | 306 |
| NPL | Total N | 2.72 (0.13) | 0.0069 | 0.0017 |  | 0.0082 | 0.0433 | 0.0007 |  | 77 | 0.18 | 5.76 | 526 |
| SPL | Total N | 2.57 (0.07) | 0.0120 | 0.0196 |  | 0.0006 |  |  |  | 42 | 0.48 | 4.30 | 370 |
| TPL | Total N | 2.46 (0.11) | 0.0077 |  |  | -0.0009 | 0.2532 |  | 0.0027 | 123 | 0.44 | 4.78 | 288 |
| UMW | Total N | 2.60 (0.06) | 0.0115 | 0.0128 |  |  |  |  |  | 52 | 0.61 | 2.86 | 397 |
| CBP | Total N | 2.52 (0.31) | 0.0106 |  |  |  | 0.1629 | 0.0216 |  | 57 | 0.22 | 3.53 | 333 |
| IN + IL | Total N | 2.30 (0.34) | 0.0134 |  | 0.0158 |  |  | 0.0303 |  | 24 | 0.34 | 4.16 | 196 |
| All | Total P | 1.43 (0.08) | 0.0058 | 0.0068 |  | 0.0002 |  | 0.0661 | 0.0041 | 306 | 0.17 | 6.19 | 26 |
| $N P L$ | Total P | 1.87 (0.29) |  |  |  | ression not si | nificant |  |  | 75 | 0.09 | na | 73 |
| SPL | Total P | 1.37 (0.12) | 0.0142 | 0.0313 |  | 0.0012 |  |  |  | 42 | 0.30 | 3.50 | 23 |
| TPL | Total P | 1.85 (0.18) |  |  |  | ression not si | ificant |  |  | 120 | 0.07 | na | 70 |
| UMW | Total P | 1.31 (0.09) | 0.0079 | 0.0160 |  |  |  | -0.0091 |  | 51 | 0.35 | 3.52 | 19 |
| CBP | Total P | 1.97 (0.50) |  |  |  | ression not si | nificant |  |  | 53 | 0.09 | na | 92 |
| IN + IL | Total P | 1.55 (0.24) |  |  |  | -0.0034 | 0.3898 |  |  | 25 | 0.20 | 2.96 | 34 |

Table 8. CTVs for total N based on interpretation of piecewise regression models. $M n=$ minimum observed nutrient concentration, $R t=$ response threshold, $S t=$ secondary threshold, $M x=$ maximum nutrient value, CTV = interpreted threshold value. See Fig. 3 and text for explanation of terms. Mn, St, or Mx listed only if used to calculate $R m$. Missing values for CTV are for relationships without reliable breakpoints. Data plotted in Appendix 6. Metric values decrease with increasing nutrients unless indicated by " $(+)$ ". Back-transformed data given in Appendix 11.

| Metric | Piecewise model interpretation based on raw metrics |  |  |  |  |  |  | Piecewise model interpretation based on regression residuals |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $r^{2}$ | Mn | Rt | St or Mx | Rm | CTV | $\begin{gathered} \text { CTV } \\ (\mathrm{ug} / \mathrm{L}) \end{gathered}$ | $r^{2}$ | Mn | Rt | St or Mx | Rm | CTV | $\begin{gathered} \text { CTV } \\ (\mathrm{ug} / \mathrm{L}) \end{gathered}$ |
| OLLEPTAX (+) | 0.15 |  | 4.08 | 4.64 | 4.36 | 4.08 | 12022 | 0.12 |  | 4.08 | 4.64 | 4.36 | 4.08 | 12022 |
| TL07RICH | 0.16 | 2.04 | 3.11 |  | 2.58 | 2.58 | 379 | 0.18 |  | 2.52 | 2.87 | 2.70 | 2.52 | 330 |
| HBI (+) | 0.14 |  | 2.9 | 2.92 | 2.91 | 2.91 | 812 | 0.09 |  | 2.39 | 3.01 | 2.70 | 2.39 | 244 |
| PLECRICH | 0.22 |  | 2.32 | 2.61 | 2.47 | 2.47 | 294 | 0.14 | 2.04 |  | 2.68 | 2.36 | 2.36 | 228 |
| ODONRICH | 0.09 |  | 2.71 | 2.81 | 2.76 | 2.71 | 512 | 0.10 |  | 2.59 | 4.64 | 3.62 | 2.59 | 388 |
| CLMBRICH | 0.08 |  | 2.52 | 4.64 | 3.58 | 2.52 | 330 | 0.12 |  | 3.39 | 4.64 | 4.02 |  |  |
| TL89PTAX (+) | 0.08 | 2.04 |  | 3.13 | 2.59 | 2.59 | 388 | 0.03 | 2.04 |  | 3.14 | 2.59 | 2.59 | 388 |
| TL01RICH | 0.25 |  | 2.3 | 2.63 | 2.47 | 2.47 | 294 | 0.21 |  | 2.28 | 2.87 | 2.58 | 2.58 | 379 |
| SHRDRICH | 0.09 | 2.04 | 2.88 |  | 2.46 | 2.46 | 287 | 0.09 | 2.04 |  | 2.93 | 2.49 | 2.49 | 308 |
| HABT_PT | 0.07 |  | 2.44 | 2.44 | 2.44 | 2.44 | 274 | 0.04 | 2.04 |  | 3.33 | 2.69 | 2.69 | 489 |
| TL67RICH | 0.06 |  | 2.5 | 3.08 | 2.79 | 2.50 | 315 | 0.08 | 2.04 |  | 3.31 | 2.68 | 2.68 | 478 |
| SCRPRICH | 0.04 | 2.04 | 3.11 |  | 2.58 | 2.58 | 379 | 0.04 | 2.04 |  | 4.64 | 3.34 |  |  |
| TOLR_PT | 0.09 | 2.04 | 3.05 |  | 2.55 | 2.55 | 354 | 0.05 | 2.04 |  | 3.01 | 2.53 | 2.53 | 338 |
| TL03PIND | 0.11 |  | 2.32 | 3.01 | 2.67 | 2.67 | 467 | 0.09 |  | 2.32 | 2.97 | 2.65 | 2.65 | 446 |
| MMI_WSABEST | 0.14 | 2.04 | 3.14 |  | 2.59 | 2.59 | 388 | 0.07 |  | 2.17 | 2.20 | 2.19 |  |  |
| CHIRRICH | 0.07 | 2.04 | 3.63 |  | 2.84 | 2.84 | 691 | 0.11 | 2.04 |  | 3.99 | 3.02 | 3.02 | 1046 |
| PREDRICH | 0.17 |  | 2.16 | 2.23 | 2.20 |  |  | 0.16 |  | 2.09 | 2.28 | 2.19 | 2.28 | 190 |
| HPRIME | 0.08 | 2.04 |  | 3.28 | 2.66 | 2.66 | 456 | 0.14 | 2.04 |  | 3.28 | 2.66 | 2.66 | 456 |
| SPRLRICH | 0.12 | 2.04 |  | 3.07 | 2.56 | 2.56 | 362 | 0.16 | 2.04 |  | 2.99 | 2.52 | 2.52 | 330 |
| INTLRICH | 0.21 |  | 2.37 | 2.63 | 2.50 | 2.50 | 315 | 0.21 |  | 2.35 | 2.87 | 2.61 | 2.61 | 406 |
| FEED_PT | 0.07 |  | 2.53 | 2.62 | 2.58 | 2.58 | 379 | 0.04 |  | 3.57 | 3.59 | 3.58 | 3.57 | 3714 |
| COGARICH | 0.09 | 2.04 |  | 3.12 | 2.58 | 2.58 | 379 | 0.12 | 2.04 |  | 4.00 | 3.02 | 3.02 | 1046 |
| EPT_RICH | 0.15 |  | 2.37 | 2.61 | 2.49 | 2.49 | 308 | 0.13 |  | 2.36 | 2.75 | 2.56 | 2.56 | 362 |
| EPHE_PT | 0.12 |  | 3.04 | 3.08 | 3.06 | 3.04 | 1095 | 0.05 | 2.04 |  | 4.64 | 3.34 |  |  |
| Median | 0.10 |  |  |  |  |  | 379 | 0.11 |  |  |  |  |  | 388 |

Table 9. CTVs for total P based on interpretation of piecewise regression models. $M n=$ minimum observed nutrient concentration, $R t=$ response threshold, $\mathrm{St}=$ secondary threshold, $\mathrm{Mx}=$ maximum nutrient value, CTV = interpreted threshold value. See Fig. 3 and text for explanation of terms. Mn, St, or Mx listed only if used to calculate $R m$. Missing values for CTV are for relationships without reliable breakpoints. Data plotted in Appendix 6. Metric values decrease with increasing nutrients unless indicated by " $(+)$ ". Back-transformed data given in Appendix 11.

| Metric | Piecewise model interpretation based on raw metrics |  |  |  |  |  |  | Piecewise model interpretation based on regression residuals |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $r^{2}$ | Mn | Rt | $S t$ or <br> Mx | Rm | CTV | $\begin{gathered} \text { CTV } \\ (\mathrm{ug} / \mathrm{L}) \\ \hline \end{gathered}$ | $r^{2}$ | Mn | $R t$ | St or Mx | Rm | CTV | $\begin{gathered} \text { CTV } \\ (\mathrm{ug} / \mathrm{L}) \end{gathered}$ |
| OLLEPTAX (+) | 0.05 | 0.48 |  | 3.73 | 2.11 |  |  | 0.06 | 0.48 |  | 3.73 | 2.11 |  |  |
| TL07RICH | 0.22 |  | 1.55 | 1.56 | 1.56 | 1.55 | 34 | 0.17 |  | 1.63 | 1.63 | 1.63 | 1.63 | 42 |
| HBI (+) | 0.09 |  | 2.75 | 3.07 | 2.91 | 2.75 | 561 | 0.05 | 0.48 |  | 3.73 | 2.11 |  |  |
| PLECRICH | 0.10 | 0.48 |  | 1.72 | 1.10 | 1.10 | 12 | 0.10 |  | 1.28 | 1.49 | 1.28 | 1.39 | 24 |
| ODONRICH | 0.05 | 0.48 |  | 2.01 | 1.25 | 1.25 | 17 | 0.06 |  | 1.78 | 1.78 | 1.78 | 1.78 | 59 |
| CLMBRICH | 0.09 |  | 1.50 | 3.73 | 2.62 | 1.50 | 31 | 0.10 |  | 1.73 | 1.79 | 1.76 | 1.76 | 57 |
| TL89PTAX (+) | 0.08 | 0.48 |  | 3.73 | 2.11 |  |  | 0.05 | 0.48 |  | 2.66 | 1.57 | 1.57 | 36 |
| TL01RICH | 0.18 |  | 1.34 | 1.34 | 1.34 | 1.34 | 21 | 0.16 | 0.48 |  | 2.02 | 1.25 | 1.25 | 17 |
| SHRDRICH | 0.11 | 0.48 |  | 1.98 | 1.23 | 1.23 | 16 | 0.09 | 0.48 |  | 2.00 | 1.24 | 1.24 | 16 |
| HABT_PT | 0.10 | 0.48 |  | 1.15 | 0.82 | 0.82 | 6 | 0.05 | 0.48 |  | 1.15 | 0.82 | 0.82 | 6 |
| TL67RICH | 0.11 |  | 1.48 | 2.49 | 1.99 | 1.48 | 29 | 0.09 |  | 1.54 | 1.79 | 1.67 | 1.54 | 34 |
| SCRPRICH | 0.10 | 0.48 |  | 3.73 | 2.11 |  |  | 0.07 | 0.48 | 2.18 |  | 1.33 | 1.33 | 20 |
| TOLR_PT | 0.07 | 0.48 |  | 3.73 | 2.11 |  |  | 0.05 |  | 0.79 | 0.90 | 0.85 | 0.9 | 7 |
| TL03PIND | 0.07 |  | 0.87 | 1.04 | 0.96 | 0.96 | 8 | 0.06 |  | 0.86 | 0.88 | 0.87 |  |  |
| MMI_WSABEST | 0.11 | 0.48 |  | 3.73 | 2.11 |  |  | 0.06 | 0.48 |  | 3.73 | 2.11 |  |  |
| CHIRRICH | 0.11 |  | 1.45 | 3.73 | 1.89 | 1.45 | 27 | 0.07 |  | 1.45 | 3.73 | 2.59 | 1.45 | 27 |
| PREDRICH | 0.11 |  | 1.27 | 3.73 | 2.50 | 1.27 | 18 | 0.06 | 0.48 |  | 1.96 | 1.22 | 1.22 | 16 |
| HPRIME | 0.14 |  | 2.57 | 3.73 | 3.15 | 2.57 | 371 | 0.10 |  | 2.78 | 3.73 | 3.26 | 2.78 | 602 |
| SPRLRICH | 0.15 |  | 1.22 | 4.64 | 2.93 | 1.22 | 16 | 0.12 | 0.48 |  | 1.98 | 1.23 | 1.23 | 16 |
| INTLRICH | 0.19 | 0.48 |  | 1.75 | 1.12 | 1.12 | 12 | 0.17 | 0.48 |  | 2.01 | 1.25 | 1.25 | 17 |
| FEED_PT | 0.08 |  | 1.61 | 1.62 | 1.62 | 1.61 | 40 | 0.06 |  | 1.61 | 1.62 | 1.62 | 1.61 | 40 |
| COGARICH | 0.16 |  | 1.21 | 3.73 | 2.47 | 1.21 | 15 | 0.12 |  | 1.00 | 3.73 | 2.37 | 1 | 9 |
| EPT_RICH | 0.13 |  | 1.00 | 1.10 | 1.05 | 1.05 | 10 | 0.09 | 0.48 |  | 1.94 | 1.21 | 1.21 | 15 |
| EPHE_PT | 0.04 | 0.48 |  | 1.69 | 1.09 | 1.09 | 11 | 0.02 | 0.48 |  | 3.73 | 2.11 |  |  |
| Median | 0.11 |  |  |  |  |  | 17 | 0.07 |  |  |  |  |  | 20 |

Table 10. CTVs for total N based on interpretation of piecewise regression models with data grouped by mean dominant substrate size. $M n=$ minimum observed nutrient concentration, $R t=$ response threshold, $S t=$ secondary threshold, $M x=$ maximum nutrient value. See Fig. 3 and text for explanation of terms. Mn, St, or Mx listed only if used to calculate Rm. Missing values for CTV are relationships without reliable breakpoints. Data plotted in Appendix 7. Metric values decrease with increasing nutrients unless indicated by " $(+)$ ". Back-transformed data given in Appendix 11.

| Metric | Piecewise model interpretation based on raw metrics for streams with fine substrates ( $<1 \mathrm{~mm}$ ) |  |  |  |  |  |  | Piecewise model interpretation base on raw metrics for streams with coarse substrates ( $\geq 1 \mathrm{~mm}$ ) |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $r^{2}$ | Mn | $R t$ | St or Mx | Rm | CTV | $\begin{gathered} \text { CTV } \\ (\mathrm{ug} / \mathrm{L}) \end{gathered}$ | $r^{2}$ | Mn | $R t$ | St or Mx | Rm | CTV | $\begin{gathered} \hline \text { CTV } \\ (\mathrm{ug} / \mathrm{L}) \\ \hline \end{gathered}$ |
| OLLEPTAX (+) | 0.13 |  | 4.08 | 4.33 | 4.21 | 4.08 | 12022 | 0.15 | 2.06 |  |  | 2.06 |  |  |
| TL07RICH | 0.16 |  | 2.52 | 2.62 | 2.57 | 2.57 | 371 | 0.18 | 2.06 | 3.23 |  | 2.65 | 2.65 | 446 |
| HBI (+) | 0.11 |  | 2.90 | 3.01 | 2.96 | 2.96 | 911 | 0.14 |  | 2.96 | 4.64 | 3.80 | 2.96 | 911 |
| PLECRICH | 0.22 |  | 2.52 | 2.53 | 2.53 | 2.53 | 338 | 0.22 |  | 2.31 | 2.50 | 2.41 | 2.41 | 256 |
| ODONRICH | 0.12 |  | 2.73 | 2.74 | 2.74 | 2.74 | 549 | 0.12 |  | 2.05 | 4.64 | 3.35 |  |  |
| CLMBRICH | 0.09 |  | 2.75 | 2.75 | 2.75 | 2.75 | 561 | 0.11 |  | 3.45 | 4.64 | 4.05 | 3.45 | 2817 |
| TL89PTAX (+) | 0.11 |  | 2.90 | 3.01 | 2.96 | 2.96 | 911 | 0.07 | 2.06 | 2.97 |  | 2.52 | 2.52 | 330 |
| TL01RICH | 0.20 | 2.04 | 2.88 |  | 2.46 | 2.46 | 287 | 0.27 |  | 2.31 | 2.48 | 2.40 | 2.40 | 250 |
| SHRDRICH | 0.07 |  | 2.54 | 2.56 | 2.55 | 2.55 | 354 | 0.21 |  | 2.24 | 3.09 | 2.67 | 2.67 | 467 |
| HABT_PT | 0.05 |  | 3.01 | 3.02 | 3.02 | 3.02 | 1046 | 0.18 | 2.06 | 3.22 |  | 2.64 | 2.64 | 436 |
| TL67RICH | 0.08 |  | 2.54 | 2.73 | 2.64 | 2.64 | 436 | 0.09 |  | 2.77 | 3.01 | 2.89 | 2.77 | 588 |
| SCRPRICH | 0.02 |  | 2.37 | 4.33 | 3.35 |  |  | 0.07 | 2.06 | 3.45 |  | 2.76 | 2.76 | 574 |
| TOLR_PT | 0.06 | 2.04 | 2.98 |  | 2.51 | 2.51 | 323 | 0.10 | 2.06 | 2.86 |  | 2.46 | 2.46 | 287 |
| TL03PIND | 0.08 |  | 2.28 | 3.12 | 2.70 | 2.70 | 500 | 0.17 | 2.06 | 3.23 |  | 2.65 | 2.65 | 446 |
| MMI_WSABEST | 0.11 | 2.04 | 3.31 |  | 2.68 | 2.68 | 478 | 0.13 |  | 2.32 | 2.69 | 2.51 | 2.51 | 323 |
| CHIRRICH | 0.09 | 2.04 | 3.01 |  | 2.53 | 2.53 | 338 | 0.06 | 2.06 | 3.93 |  | 3.00 |  |  |
| PREDRICH | 0.12 | 2.04 | 2.75 |  | 2.40 |  |  | 0.07 | 2.06 |  |  | 2.06 |  |  |
| HPRIME | 0.06 | 2.04 | 3.13 |  | 2.59 | 2.59 | 388 | 0.07 |  | 2.59 | 3.12 | 2.86 | 2.59 | 388 |
| SPRLRICH | 0.09 | 2.04 | 2.96 |  | 2.50 | 2.50 | 315 | 0.12 |  | 2.29 | 3.02 | 2.66 | 2.66 | 456 |
| INTLRICH | 0.16 |  | 2.58 | 2.58 | 2.58 | 2.58 | 379 | 0.19 | 2.06 | 3.22 |  | 2.64 | 2.64 | 436 |
| FEED_PT | 0.06 |  | 2.39 | 2.75 | 2.57 | 2.57 | 371 | 0.08 | 2.06 | 2.12 |  | 2.09 |  |  |
| COGARICH | 0.10 | 2.04 | 3.00 |  | 2.52 | 2.52 | 330 | 0.07 |  | 2.59 | 3.13 | 2.86 | 2.59 | 388 |
| EPT_RICH | 0.11 |  | 2.43 | 2.63 | 2.53 | 2.53 | 338 | 0.13 | 2.06 | 3.28 |  | 2.67 | 2.67 | 467 |
| EPHE_PT | 0.10 |  | 2.90 | 3.30 | 3.10 | 3.10 | 1258 | 0.09 | 2.06 | 2.11 |  | 2.09 |  |  |
| Median | 0.10 |  |  |  |  |  | 384 | 0.12 |  |  |  |  |  | 441 |

Table 11. CTVs for total $P$ based on interpretation of piecewise regression models with data grouped by mean dominant substrate size. $M n=$ minimum observed nutrient concentration, $R t=$ response threshold, $S t=$ secondary threshold, $M x=$ maximum nutrient value. See Fig. 3 and text for explanation of terms. Mn, St, or $M x$ listed only if used to calculate $R m$. Missing values for CTV are for relationships without reliable breakpoints. Data plotted in Appendix 7. Metric values decrease with increasing nutrients unless indicated by " $(+)$ ". Back-transformed data given in Appendix 11.

| Metric | Piecewise model interpretation based on raw metrics for streams with fine substrates ( $<1 \mathrm{~mm}$ ) |  |  |  |  |  |  | Piecewise model interpretation base on raw metrics for streams with coarse substrates ( $\geq 1 \mathrm{~mm}$ ) |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $r^{2}$ | Mn | $R t$ | St or Mx | Rm | CTV | $\begin{gathered} \text { CTV } \\ (\mathrm{ug} / \mathrm{L}) \end{gathered}$ | $r^{2}$ | Mn | $R t$ | St or Mx | Rm | CTV | $\begin{gathered} \hline \text { CTV } \\ (\mathrm{ug} / \mathrm{L}) \end{gathered}$ |
| OLLEPTAX (+) | 0.13 | 0.48 | 3.61 |  | 2.05 |  |  | 0.16 |  | 1.86 | 2.74 | 2.30 | 1.86 | 71 |
| TL07RICH | 0.15 |  | 1.52 | 1.67 | 1.60 | 1.60 | 39 | 0.35 |  | 1.51 | 2.74 | 2.13 | 1.51 | 31 |
| HBI (+) | 0.07 |  | 2.75 | 3.06 | 2.91 | 2.75 | 561 | 0.17 | 0.61 | 2.15 |  | 1.38 | 1.38 | 23 |
| PLECRICH | 0.07 | 0.48 | 1.42 |  | 0.95 | 0.95 | 8 | 0.31 |  | 1.30 | 1.31 | 1.31 | 1.31 | 19 |
| ODONRICH | 0.08 | 0.48 | 2.63 |  | 1.56 | 1.56 | 35 | 0.06 | 0.61 | 2.01 |  | 1.31 | 1.31 | 19 |
| CLMBRICH | 0.10 |  | 1.32 | 3.73 | 2.53 | 1.32 | 20 | 0.09 | 0.61 | 0.87 |  | 0.74 |  |  |
| TL89PTAX (+) | 0.07 |  | 1.08 | 3.35 | 2.22 |  |  | 0.15 | 0.61 | 2.28 |  | 1.45 | 1.45 | 27 |
| TL01RICH | 0.20 |  | 0.93 | 1.01 | 0.97 | 0.97 | 8 | 0.20 |  | 1.06 | 2.74 | 1.90 | 1.06 | 10 |
| SHRDRICH | 0.08 | 0.48 | 1.91 |  | 1.20 | 1.20 | 15 | 0.20 | 0.61 | 2.37 |  | 1.49 | 1.49 | 30 |
| HABT_PT | 0.06 | 0.48 | 3.19 |  | 1.84 |  |  | 0.15 | 0.61 | 0.64 |  | 0.63 |  |  |
| TL67RICH | 0.11 |  | 1.67 | 1.72 | 1.70 | 1.70 | 49 | 0.25 |  | 1.33 | 1.42 | 1.38 | 1.42 | 25 |
| SCRPRICH | 0.07 |  | 1.31 | 3.73 | 2.52 | 1.31 | 19 | 0.13 |  | 0.87 | 0.89 | 0.88 |  |  |
| TOLR_PT | 0.04 |  | 1.21 | 3.73 | 2.47 | 1.21 | 15 | 0.17 |  | 2.51 | 2.74 | 2.63 |  |  |
| TL03PIND | 0.03 |  | 2.75 | 3.73 | 3.24 | 2.75 | 561 | 0.11 |  | 1.03 | 2.74 | 1.89 | 1.03 | 10 |
| MMI_WSABEST | 0.07 |  | 2.31 | 3.73 | 3.02 | 2.31 | 203 | 0.23 | 0.61 | 2.26 |  | 1.44 | 1.44 | 27 |
| CHIRRICH | 0.11 |  | 1.05 | 1.13 | 1.09 |  |  | 0.34 |  | 1.76 | 2.74 | 2.25 | 1.76 | 57 |
| PREDRICH | 0.06 |  | 2.88 | 3.73 | 3.31 |  |  | 0.23 |  | 1.04 | 2.74 | 1.89 | 1.04 | 10 |
| HPRIME | 0.12 |  | 2.59 | 3.73 | 3.16 | 2.59 | 388 | 0.19 |  | 1.51 | 2.74 | 2.13 | 1.51 | 31 |
| SPRLRICH | 0.12 |  | 1.04 | 1.12 | 1.08 |  |  | 0.35 |  | 1.56 | 2.74 | 2.15 | 1.56 | 35 |
| INTLRICH | 0.16 |  | 0.97 | 0.97 | 0.97 |  |  | 0.29 |  | 1.00 | 2.74 | 1.87 | 1.00 | 9 |
| FEED_PT | 0.06 | 0.48 |  | 3.73 | 2.11 |  |  | 0.10 | 0.61 | 2.26 |  | 1.44 | 1.44 | 27 |
| COGARICH | 0.14 |  | 1.21 | 3.73 | 1.81 | 1.21 | 15 | 0.25 |  | 1.56 | 2.74 | 2.15 | 1.56 | 35 |
| EPT_RICH | 0.09 |  | 0.98 | 0.98 | 0.98 |  |  | 0.22 |  | 1.06 | 1.66 | 1.36 | 1.36 | 22 |
| EPHE_PT | 0.02 | 0.48 | 0.00 | 3.73 | 1.40 |  |  | 0.06 | 0.61 |  | 2.74 | 1.68 |  |  |
| Median | 0.08 |  |  |  |  |  | 28 | 0.20 |  |  |  |  |  | 27 |

Table 12. Candidate threshold values (CTVs) for total N based on interpolation of linear regression models. CTV determined by interpolating the total N value corresponding to the median metric value at reference sites. Data plotted in Appendix 8. Asterisk indicates a reference value (47.6) from Herlihy and Sifneos (2008). Metric values decrease with increasing nutrients unless indicated by "(+)".

| Metric | $25^{\text {th }}$ percentile |  | $75^{\text {th }}$ percentile |  | Median |  | $r^{2}$ | CTV | $\begin{gathered} \text { CTV } \\ (\mathrm{ug} / \mathrm{L}) \end{gathered}$ | 50\% prediction limits (ug/L) |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Nonreference | Reference | Nonreference | Reference | Nonreference | Reference |  |  |  |  |  |
| OLLEPTAX (+) | 2.9 | 2.5 | 7.0 | 4.0 | 4.3 | 3.2 | 0.12 | 2.38 | 238 | 2208 | 25 |
| TL07RICH | 16.0 | 23.0 | 28.0 | 33.0 | 22.0 | 27.0 | 0.12 | 2.47 | 297 | 32 | 2688 |
| HBI (+) | 5.2 | 4.6 | 6.6 | 5.7 | 5.8 | 5.3 | 0.06 | 2.23 | 167 | 4553 | 5 |
| PLECRICH | 0.0 | 0.0 | 0.0 | 2.0 | 0.0 | 0.0 | 0.09 | 3.75 | 5570 | 425 | 73941 |
| ODONRICH | 0.0 | 0.0 | 1.0 | 1.0 | 1.0 | 1.0 | 0.07 | 2.78 | 606 | 31 | 11584 |
| CLMBRICH | 3.0 | 3.0 | 5.0 | 5.0 | 4.0 | 4.0 | 0.07 | 3.20 | 1568 | 85 | 28854 |
| TL89PTAX (+) | 13.8 | 11.1 | 25.0 | 20.5 | 20.0 | 15.1 | 0.05 | 1.94 | 85 | 3384 | 1 |
| TL01RICH | 0.0 | 0.0 | 1.0 | 3.0 | 0.0 | 1.0 | 0.14 | 3.04 | 1086 | 141 | 8357 |
| SHRDRICH | 2.0 | 3.0 | 5.0 | 6.0 | 4.0 | 4.0 | 0.05 | 3.00 | 1005 | 29 | 33248 |
| HABT_PT | 1.7 | 3.7 | 5.3 | 6.7 | 3.6 | 5.1 | 0.04 | 1.89 | 77 | 1 | 3420 |
| TL67RICH | 5.0 | 7.0 | 10.0 | 10.0 | 8.0 | 8.0 | 0.06 | 3.00 | 1001 | 35 | 27903 |
| SCRPRICH | 2.0 | 3.0 | 5.0 | 6.0 | 3.0 | 4.0 | 0.03 | 2.54 | 347 | 2 | 49102 |
| TOLR_PT | 1.3 | 3.9 | 5.7 | 8.5 | 3.4 | 5.9 | 0.02 | 0.46 | 2 | -1 | 1028 |
| TL03PIND | 2.7 | 9.7 | 23.3 | 33.3 | 9.0 | 20.3 | 0.02 | 2.26 | 180 | 0 | 36715 |
| MMI_WSABEST | 21.8 | 35.7 | 45.3 | 65.0 | 33.4 | 50.5 | 0.10 | 1.93 | 84 | 6 | 1004 |
| MMI_WSABEST* |  |  |  |  |  | 47.6 | 0.10 | 2.19 | 153 | 12 | 1820 |
| CHIRRICH | 9.0 | 10.0 | 16.0 | 15.0 | 12.0 | 13.0 | 0.04 | 2.88 | 750 | 11 | 48146 |
| PREDRICH | 5.0 | 6.0 | 11.0 | 13.0 | 8.0 | 10.0 | 0.15 | 2.58 | 384 | 56 | 2591 |
| HPRIME | 1.9 | 2.2 | 2.7 | 2.8 | 2.4 | 2.6 | 0.04 | 1.97 | 93 | 1 | 5072 |
| SPRLRICH | 6.0 | 9.0 | 12.0 | 14.0 | 9.0 | 10.0 | 0.08 | 2.94 | 874 | 60 | 12662 |
| INTLRICH | 1.0 | 2.0 | 5.0 | 8.0 | 3.0 | 4.0 | 0.09 | 3.02 | 1037 | 78 | 13706 |
| FEED_PT | 1.3 | 2.5 | 5.0 | 7.1 | 2.9 | 5.4 | 0.06 | 1.57 | 36 | 0 | 998 |
| COGARICH | 10.0 | 12.0 | 17.0 | 18.0 | 14.0 | 15.0 | 0.05 | 2.66 | 454 | 15 | 13542 |
| EPT_RICH | 2.0 | 4.0 | 7.0 | 11.0 | 4.0 | 7.0 | 0.08 | 2.52 | 332 | 21 | 4993 |
| EPHE_PT | 1.0 | 1.4 | 4.3 | 7.1 | 2.0 | 3.9 | 0.09 | 2.63 | 429 | 34 | 5306 |
| Median |  |  |  |  |  |  | 0.07 |  | 347 | 31 | 5306 |

Table 13. Candidate threshold values (CTVs) for total P based on interpolation of linear regression models. CTV determined by interpolating the total N value corresponding to the median metric value at reference sites. Data plotted in Appendix 8. Asterisk indicates a reference value (47.6) from Herlihy and Sifneos (2008). Metric values decrease with increasing nutrients unless indicated by "(+)".

| Metric | $25^{\text {th }}$ percentile |  | $75^{\text {th }}$ percentile |  | Median |  | $r^{2}$ | CTV | $\begin{aligned} & \text { CTV } \\ & (\mathrm{ug} / \mathrm{L}) \end{aligned}$ | 50\% prediction limits (ug/L) |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Nonreference | Reference | Nonreference | Reference | Nonreference | Reference |  |  |  |  |  |
| OLLEPTAX (+) | 2.9 | 2.5 | 7.0 | 4.0 | 4.3 | 3.2 | 0.04 | 0.58 | 3 | 251 | -1 |
| TL07RICH | 16.0 | 23.0 | 28.0 | 33.0 | 22.0 | 27.0 | 0.20 | 1.38 | 23 | 3 | 142 |
| HBI (+) | 5.2 | 4.6 | 6.6 | 5.7 | 5.8 | 5.3 | 0.06 | 1.00 | 9 | 309 | -1 |
| PLECRICH | 0.0 | 0.0 | 0.0 | 2.0 | 0.0 | 0.0 | 0.08 | 2.64 | 436 | 22 | 8202 |
| ODONRICH | 0.0 | 0.0 | 1.0 | 1.0 | 1.0 | 1.0 | 0.05 | 1.47 | 29 | 0 | 1674 |
| CLMBRICH | 3.0 | 3.0 | 5.0 | 5.0 | 4.0 | 4.0 | 0.09 | 2.01 | 100 | 5 | 1851 |
| TL89PTAX (+) | 13.8 | 11.1 | 25.0 | 20.5 | 20.0 | 15.1 | 0.07 | 0.86 | 6 | 191 | -1 |
| TL01RICH | 0.0 | 0.0 | 1.0 | 3.0 | 0.0 | 1.0 | 0.14 | 1.84 | 68 | 7 | 629 |
| SHRDRICH | 2.0 | 3.0 | 5.0 | 6.0 | 4.0 | 4.0 | 0.10 | 1.84 | 68 | 4 | 1005 |
| HABT_PT | 1.7 | 3.7 | 5.3 | 6.7 | 3.6 | 5.1 | 0.06 | 0.81 | 5 | -1 | 195 |
| TL67RICH | 5.0 | 7.0 | 10.0 | 10.0 | 8.0 | 8.0 | 0.09 | 1.83 | 66 | 3 | 1170 |
| SCRPRICH | 2.0 | 3.0 | 5.0 | 6.0 | 3.0 | 4.0 | 0.09 | 1.59 | 38 | 1 | 649 |
| TOLR_PT | 1.3 | 3.9 | 5.7 | 8.5 | 3.4 | 5.9 | 0.07 | 0.43 | 2 | -1 | 67 |
| TL03PIND | 2.7 | 9.7 | 23.3 | 33.3 | 9.0 | 20.3 | 0.06 | 1.33 | 21 | 0 | 792 |
| MMI_WSABEST | 21.8 | 35.7 | 45.3 | 65.0 | 33.4 | 50.5 | 0.11 | 0.72 | 4 | -1 | 62 |
| MMI_WSABEST* |  |  |  |  |  | 47.6 | 0.11 | 0.98 | 9 | 0 | 114 |
| CHIRRICH | 9.0 | 10.0 | 16.0 | 15.0 | 12.0 | 13.0 | 0.09 | 1.76 | 57 | 3 | 934 |
| PREDRICH | 5.0 | 6.0 | 11.0 | 13.0 | 8.0 | 10.0 | 0.11 | 1.22 | 16 | 0 | 221 |
| HPRIME | 1.9 | 2.2 | 2.7 | 2.8 | 2.4 | 2.6 | 0.13 | 1.22 | 16 | 1 | 173 |
| SPRLRICH | 6.0 | 9.0 | 12.0 | 14.0 | 9.0 | 10.0 | 0.15 | 1.78 | 60 | 6 | 510 |
| INTLRICH | 1.0 | 2.0 | 5.0 | 8.0 | 3.0 | 4.0 | 0.18 | 1.85 | 70 | 9 | 488 |
| FEED_PT | 1.3 | 2.5 | 5.0 | 7.1 | 2.9 | 5.4 | 0.07 | 0.43 | 2 | -1 | 63 |
| COGARICH | 10.0 | 12.0 | 17.0 | 18.0 | 14.0 | 15.0 | 0.15 | 1.62 | 41 | 4 | 361 |
| EPT_RICH | 2.0 | 4.0 | 7.0 | 11.0 | 4.0 | 7.0 | 0.11 | 1.35 | 21 | 1 | 298 |
| EPHE_PT | 1.0 | 1.4 | 4.3 | 7.1 | 2.0 | 3.9 | 0.04 | 1.11 | 12 | -1 | 1011 |
| Median |  |  |  |  |  |  | 0.09 |  | 21 | 3 | 361 |

Table 14. Candidate threshold values (CTVs) for total N for streams with fine substrate based on interpolation of linear regression models. CTV determined by interpolating the total N value corresponding to the median metric value at reference sites. Data plotted in Appendix 9. Asterisk indicates an adjusted reference value (47.6) from Herlihy and Sifneos (2008) for MMI_WSABEST.

| Metric | $25^{\text {th }}$ percentile |  | $75^{\text {th }}$ percentile |  | Median |  | $r^{2}$ | CTV | $\begin{aligned} & \text { CTV } \\ & (\mathrm{ug} / \mathrm{L}) \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Nonreference | Reference | Nonreference | Reference | Nonreference | Reference |  |  |  |
| OLLEPTAX (+) | 2.9 | 2.4 | 7.1 | 5.6 | 4.3 | 3.7 | 0.10 | 2.56 | 365 |
| TL07RICH | 14.0 | 22.0 | 27.0 | 35.0 | 21.0 | 27.0 | 0.10 | 2.22 | 164 |
| HBI (+) | 5.3 | 4.8 | 6.7 | 5.9 | 5.9 | 5.5 | 0.03 | 2.05 | 112 |
| PLECRICH | 0.0 | 0.0 | 0.0 | 2.0 | 0.0 | 0.0 | 0.05 | 3.87 | 7369 |
| ODONRICH | 0.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 0.09 | 2.99 | 968 |
| CLMBRICH | 3.0 | 3.0 | 5.0 | 5.0 | 4.0 | 4.0 | 0.09 | 3.32 | 2065 |
| TL89PTAX (+) | 13.8 | 12.5 | 27.3 | 20.7 | 20.0 | 16.7 | 0.04 | 2.13 | 135 |
| TL01RICH | 0.0 | 1.0 | 1.0 | 3.0 | 0.0 | 1.0 | 0.12 | 2.87 | 746 |
| SHRDRICH | 2.0 | 3.0 | 5.0 | 6.0 | 4.0 | 6.0 | 0.03 | -0.04 | 0 |
| HABT_PT | 1.2 | 3.5 | 5.0 | 5.8 | 3.5 | 4.7 | 0.03 | 1.71 | 50 |
| TL67RICH | 5.0 | 7.0 | 10.0 | 11.0 | 7.0 | 8.0 | 0.07 | 3.00 | 1003 |
| SCRPRICH | 2.0 | 1.0 | 4.0 | 5.0 | 3.0 | 4.0 | 0.01 | 1.18 | 14 |
| TOLR_PT | 1.3 | 3.8 | 5.6 | 7.9 | 3.3 | 4.6 | 0.01 | 0.68 | 4 |
| TL03PIND | 2.0 | 9.7 | 19.7 | 37.7 | 7.0 | 14.7 | 0.01 | 2.72 | 524 |
| MMI_WSABEST | 20.3 | 31.2 | 41.4 | 54.6 | 31.5 | 43.9 | 0.06 | 1.82 | 66 |
| MMI_WSABEST* |  |  |  |  |  | 47.6 | 0.06 | 1.35 | 21 |
| CHIRRICH | 8.0 | 11.0 | 15.0 | 20.0 | 12.0 | 15.0 | 0.04 | 1.89 | 76 |
| PREDRICH | 5.0 | 6.0 | 10.0 | 12.0 | 7.0 | 10.0 | 0.12 | 2.34 | 219 |
| HPRIME | 1.8 | 2.0 | 2.7 | 2.9 | 2.4 | 2.6 | 0.03 | 1.62 | 41 |
| SPRLRICH | 6.0 | 9.0 | 11.0 | 15.0 | 9.0 | 10.0 | 0.07 | 2.84 | 698 |
| INTLRICH | 1.0 | 2.0 | 4.0 | 7.0 | 2.0 | 3.0 | 0.05 | 3.39 | 2459 |
| FEED_PT | 1.3 | 0.0 | 4.3 | 7.1 | 2.5 | 5.7 | 0.03 | 0.15 | 0 |
| COGARICH | 10.0 | 14.0 | 16.0 | 21.0 | 13.0 | 17.0 | 0.06 | 1.83 | 67 |
| EPT_RICH | 2.0 | 3.0 | 6.0 | 11.0 | 4.0 | 5.0 | 0.05 | 3.03 | 1079 |
| EPHE_PT | 1.0 | 1.4 | 4.0 | 6.3 | 1.4 | 2.0 | 0.07 | 3.66 | 4588 |
| Median |  |  |  |  |  |  | 0.05 |  | 164 |

Table 15. Candidate threshold values (CTVs) for total N for streams with coarse substrate based on interpolation of linear regression models. CTV determined by interpolating the total N value corresponding to the median metric value at reference sites. Data plotted in Appendix 9. Asterisk indicates an adjusted reference value (47.6) from Herlihy and Sifneos (2008) for MMI_WSABEST.

| Metric | $25^{\text {th }}$ percentile |  | $75^{\text {th }}$ percentile |  | Median |  | $r^{2}$ | CTV | $\begin{aligned} & \text { CTV } \\ & (\mathrm{ug} / \mathrm{L}) \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Nonreference | Reference | Nonreference | Reference | Nonreference | Reference |  |  |  |
| OLLEPTAX (+) | 3.1 | 2.5 | 6.5 | 3.7 | 4.3 | 3.1 | 0.15 | 2.33 | 213 |
| TL07RICH | 21.0 | 23.0 | 30.0 | 33.0 | 26.0 | 27.0 | 0.08 | 2.84 | 695 |
| HBI (+) | 5.0 | 4.4 | 6.0 | 5.6 | 5.5 | 4.9 | 0.07 | 2.11 | 128 |
| PLECRICH | 0.0 | 0.0 | 1.0 | 2.0 | 0.0 | 0.0 | 0.11 | 3.73 | 5323 |
| ODONRICH | 0.0 | 0.0 | 1.0 | 1.0 | 1.0 | 1.0 | 0.07 | 2.37 | 236 |
| CLMBRICH | 3.0 | 3.0 | 5.0 | 5.0 | 4.0 | 4.0 | 0.08 | 2.86 | 727 |
| TL89PTAX (+) | 12.3 | 10.0 | 23.4 | 20.5 | 18.8 | 13.8 | 0.04 | 1.76 | 57 |
| TL01RICH | 0.0 | 0.0 | 1.0 | 3.0 | 0.0 | 1.0 | 0.15 | 3.11 | 1291 |
| SHRDRICH | 2.0 | 3.0 | 5.0 | 6.0 | 4.0 | 4.0 | 0.12 | 2.96 | 906 |
| HABT_PT | 2.6 | 4.2 | 6.2 | 7.1 | 4.5 | 5.9 | 0.02 | 1.24 | 17 |
| TL67RICH | 6.5 | 6.0 | 10.0 | 10.0 | 8.0 | 8.0 | 0.02 | 3.29 | 1946 |
| SCRPRICH | 3.0 | 3.0 | 6.0 | 6.0 | 4.0 | 5.0 | 0.01 | 1.79 | 60 |
| TOLR_PT | 1.5 | 3.9 | 5.8 | 8.6 | 3.7 | 6.9 | 0.03 | 0.66 | 4 |
| TL03PIND | 7.3 | 9.7 | 30.0 | 34.3 | 15.5 | 23.3 | 0.17 | 2.70 | 500 |
| MMI_WSABEST | 29.5 | 39.7 | 60.0 | 68.9 | 42.3 | 52.4 | 0.08 | 2.40 | 251 |
| MMI_WSABEST* | 10.0 |  |  |  |  | 47.6 | 0.08 | 2.82 | 653 |
| CHIRRICH | 6.0 | 10.0 | 16.0 | 14.0 | 14.0 | 12.0 | 0.01 | 4.14 | 13701 |
| PREDRICH | 2.2 | 8.0 | 12.0 | 14.0 | 10.0 | 10.0 | 0.14 | 2.91 | 811 |
| HPRIME | 8.0 | 2.2 | 2.9 | 2.8 | 2.6 | 2.6 | 0.02 | 2.12 | 130 |
| SPRLRICH | 2.0 | 9.0 | 13.0 | 14.0 | 10.0 | 10.0 | 0.09 | 3.16 | 1428 |
| INTLRICH | 2.0 | 2.0 | 6.0 | 9.0 | 4.0 | 4.0 | 0.09 | 3.40 | 2511 |
| FEED_PT | 11.0 | 2.9 | 6.3 | 7.1 | 4.0 | 5.0 | 0.06 | 2.46 | 286 |
| COGARICH | 4.0 | 12.0 | 18.0 | 16.0 | 15.0 | 14.0 | 0.02 | 3.45 | 2822 |
| EPT_RICH | 1.7 | 5.0 | 10.0 | 12.0 | 7.0 | 8.0 | 0.06 | 2.77 | 593 |
| EPHE_PT | 1.0 | 2.0 | 5.7 | 7.1 | 3.0 | 4.3 | 0.07 | 2.80 | 636 |
| Median |  |  |  |  |  |  | 0.07 |  | 636 |

Table 16. Candidate threshold values (CTVs) for total P for streams with fine substrate based on interpolation of linear regression models. CTV determined by interpolating the total N value corresponding to the median metric value at reference sites. Data plotted in Appendix 9. Asterisk indicates an adjusted reference value (47.6) from Herlihy and Sifneos (2008) for MMI_WSABEST.

| Metric | $25^{\text {th }}$ percentile |  | $75^{\text {th }}$ percentile |  | Median |  | $r 2$ | CTV | $\begin{gathered} \text { CTV } \\ (\mathrm{ug} / \mathrm{L}) \end{gathered}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Nonreference | Reference | Nonreference | Reference | Nonreference | Reference |  |  |  |
| OLLEPTAX (+) | 2.9 | 2.4 | 7.1 | 5.6 | 4.3 | 3.7 | 0.02 | 0.33 | 1 |
| TL07RICH | 14.0 | 22.0 | 27.0 | 35.0 | 21.0 | 27.0 | 0.14 | 1.05 | 10 |
| HBI (+) | 5.3 | 4.8 | 6.7 | 5.9 | 5.9 | 5.5 | 0.03 | 0.80 | 5 |
| PLECRICH | 0.0 | 0.0 | 0.0 | 2.0 | 0.0 | 0.0 | 0.04 | 2.82 | 658 |
| ODONRICH | 0.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 | 0.06 | 1.73 | 53 |
| CLMBRICH | 3.0 | 3.0 | 5.0 | 5.0 | 4.0 | 4.0 | 0.10 | 2.13 | 133 |
| TL89PTAX (+) | 13.8 | 12.5 | 27.3 | 20.7 | 20.0 | 16.7 | 0.04 | 0.89 | 7 |
| TL01RICH | 0.0 | 1.0 | 1.0 | 3.0 | 0.0 | 1.0 | 0.12 | 1.64 | 42 |
| SHRDRICH | 2.0 | 3.0 | 5.0 | 6.0 | 4.0 | 6.0 | 0.08 | -0.29 | 0 |
| HABT_PT | 1.2 | 3.5 | 5.0 | 5.8 | 3.5 | 4.7 | 0.02 | -0.06 | 0 |
| TL67RICH | 5.0 | 7.0 | 10.0 | 11.0 | 7.0 | 8.0 | 0.08 | 1.79 | 61 |
| SCRPRICH | 2.0 | 1.0 | 4.0 | 5.0 | 3.0 | 4.0 | 0.06 | 1.04 | 10 |
| TOLR_PT | 1.3 | 3.8 | 5.6 | 7.9 | 3.3 | 4.6 | 0.03 | 0.87 | 6 |
| TL03PIND | 2.0 | 9.7 | 19.7 | 37.7 | 7.0 | 14.7 | 0.11 | 2.39 | 246 |
| MMI_WSABEST | 20.3 | 31.2 | 41.4 | 54.6 | 31.5 | 43.9 | 0.06 | 0.49 | 2 |
| MMI_WSABEST* |  |  |  |  |  | 47.6 | 0.06 | -0.02 | 0 |
| CHIRRICH | 8.0 | 11.0 | 15.0 | 20.0 | 12.0 | 15.0 | 0.08 | 0.92 | 7 |
| PREDRICH | 5.0 | 6.0 | 10.0 | 12.0 | 7.0 | 10.0 | 0.06 | 0.60 | 3 |
| HPRIME | 1.8 | 2.0 | 2.7 | 2.9 | 2.4 | 2.6 | 0.10 | 0.95 | 8 |
| SPRLRICH | 6.0 | 9.0 | 11.0 | 15.0 | 9.0 | 10.0 | 0.10 | 1.67 | 45 |
| INTLRICH | 1.0 | 2.0 | 4.0 | 7.0 | 2.0 | 3.0 | 0.11 | 2.15 | 139 |
| FEED_PT | 1.3 | 0.0 | 4.3 | 7.1 | 2.5 | 5.7 | 0.06 | -0.46 | -1 |
| COGARICH | 10.0 | 14.0 | 16.0 | 21.0 | 13.0 | 17.0 | 0.13 | 0.98 | 9 |
| EPT_RICH | 2.0 | 3.0 | 6.0 | 11.0 | 4.0 | 5.0 | 0.06 | 1.81 | 64 |
| EPHE_PT | 1.0 | 1.4 | 4.0 | 6.3 | 1.4 | 2.0 | 0.02 | 3.17 | 1461 |
| Median |  |  |  |  |  |  | 0.06 |  | 8.6 |

Table 17. Candidate threshold values (CTVs) for total $P$ for streams with coarse substrate based on interpolation of linear regression models. CTV determined by interpolating the total N value corresponding to the median metric value at reference sites. Data plotted in Appendix 9. Asterisk indicates an adjusted reference value (47.6) from Herlihy and Sifneos (2008) for MMI_WSABEST.

| Metric | $25^{\text {th }}$ percentile |  | $75^{\text {th }}$ percentile |  | Median |  | $r^{2}$ | CTV | $\begin{gathered} \text { CTV } \\ (\mathrm{ug} / \mathrm{L}) \end{gathered}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Nonreference | Reference | Nonreference | Reference | Nonreference | Reference |  |  |  |
| OLLEPTAX (+) | 3.1 | 2.5 | 6.5 | 3.7 | 4.3 | 3.1 | 0.15 | 1.15 | 13 |
| TL07RICH | 21.0 | 23.0 | 30.0 | 33.0 | 26.0 | 27.0 | 0.30 | 1.72 | 51 |
| HBI (+) | 5.0 | 4.4 | 6.0 | 5.6 | 5.5 | 4.9 | 0.10 | 1.08 | 11 |
| PLECRICH | 0.0 | 0.0 | 1.0 | 2.0 | 0.0 | 0.0 | 0.14 | 2.47 | 294 |
| ODONRICH | 0.0 | 0.0 | 1.0 | 1.0 | 1.0 | 1.0 | 0.03 | 0.86 | 6 |
| CLMBRICH | 3.0 | 3.0 | 5.0 | 5.0 | 4.0 | 4.0 | 0.08 | 1.70 | 49 |
| TL89PTAX (+) | 12.3 | 10.0 | 23.4 | 20.5 | 18.8 | 13.8 | 0.12 | 1.08 | 11 |
| TL01RICH | 0.0 | 0.0 | 1.0 | 3.0 | 0.0 | 1.0 | 0.18 | 1.93 | 83 |
| SHRDRICH | 2.0 | 3.0 | 5.0 | 6.0 | 4.0 | 4.0 | 0.18 | 1.79 | 60 |
| HABT_PT | 2.6 | 4.2 | 6.2 | 7.1 | 4.5 | 5.9 | 0.14 | 1.18 | 14 |
| TL67RICH | 6.5 | 6.0 | 10.0 | 10.0 | 8.0 | 8.0 | 0.09 | 1.91 | 81 |
| SCRPRICH | 3.0 | 3.0 | 6.0 | 6.0 | 4.0 | 5.0 | 0.09 | 1.42 | 25 |
| TOLR_PT | 1.5 | 3.9 | 5.8 | 8.6 | 3.7 | 6.9 | 0.15 | 0.82 | 6 |
| TL03PIND | 7.3 | 9.7 | 30.0 | 34.3 | 15.5 | 23.3 | 0.11 | 1.67 | 46 |
| MMI_WSABEST | 29.5 | 39.7 | 60.0 | 68.9 | 42.3 | 52.4 | 0.16 | 1.38 | 23 |
| MMI_WSABEST* | 10.0 |  |  |  |  | 47.6 | 0.16 | 1.68 | 47 |
| CHIRRICH | 6.0 | 10.0 | 16.0 | 14.0 | 14.0 | 12.0 | 0.11 | 2.16 | 144 |
| PREDRICH | 2.2 | 8.0 | 12.0 | 14.0 | 10.0 | 10.0 | 0.18 | 1.74 | 54 |
| HPRIME | 8.0 | 2.2 | 2.9 | 2.8 | 2.6 | 2.6 | 0.15 | 1.48 | 29 |
| SPRLRICH | 2.0 | 9.0 | 13.0 | 14.0 | 10.0 | 10.0 | 0.30 | 1.89 | 76 |
| INTLRICH | 2.0 | 2.0 | 6.0 | 9.0 | 4.0 | 4.0 | 0.27 | 2.03 | 107 |
| FEED_PT | 11.0 | 2.9 | 6.3 | 7.1 | 4.0 | 5.0 | 0.05 | 1.23 | 16 |
| COGARICH | 4.0 | 12.0 | 18.0 | 16.0 | 15.0 | 14.0 | 0.15 | 1.97 | 92 |
| EPT_RICH | 1.7 | 5.0 | 10.0 | 12.0 | 7.0 | 8.0 | 0.15 | 1.66 | 45 |
| EPHE_PT | 1.0 | 2.0 | 5.7 | 7.1 | 3.0 | 4.3 | 0.06 | 1.62 | 41 |
| Median |  |  |  |  |  |  | 0.15 |  | 46 |



Figure 1. Locations of the 327 Plains/Upper Midwest sites and their watersheds used in this analysis. Portions of watersheds in Canada are ignored.


Figure 2. WSA aggregate ecoregions. Aggregate ecoregions are combined level 3 ecoregions. Figure is from http://www.epa.gov/owow/streamsurvey/.


Fig. 3. Hypothesized expected three- and two-breakpoint metric responses to increasing nutrient concentration. $M n=$ minimum observed concentration; $R t=$ response threshold, $R m=$ response midpoint, $S t=$ secondary threshold, $M x=$ maximum observed concentration. See text for additional details


| Variable | PC1 | PC2 |
| :--- | ---: | ---: |
| Riparian disturbance | 0.39 | 0.35 |
| Row crops | 0.53 | -0.21 |
| Pasture/hay | 0.28 | -0.56 |
| Development | 0.41 | -0.44 |
| Forest | -0.44 | -0.40 |
| Wetland | -0.34 | -0.40 |

Figure 4. Principal coordinates ordination and eigenvectors for land use and riparian disturbance at Plains/Upper Midwest sites. Symbols indicate WSA aggregate ecoregions.


Figure 5. Plot A) Piecewise 3 segment linear regression relationship between a human disturbance gradient (PC 1) and total N (simple linear fit shown for comparison). Plot B) Linear regression relationship between a human disturbance gradient (PC 1) and total P. Variables given in Fig. 4. Symbols indicate WSA aggregate ecoregions.


Fig. 6. Relationships between macroinvertebrate metrics and substrate size. Value of zero on $x$-axis $=$ 1 mm .


Fig. 6, continued. Relationships between macroinvertebrate metrics and substrate size. Value of zero on x -axis $=1 \mathrm{~mm}$.


Fig. 6, continued. Relationships between macroinvertebrate metrics and substrate size. Value of zero on x -axis $=1 \mathrm{~mm}$.


Fig. 7. Candidate threshold values (CTV) plotted against $r^{2}$ for the applicable piecewise regression (data from Tables 8 and 9). Horizontal lines at 100 and $1000 \mathrm{ug} / \mathrm{L}$ are for reference. CTVs < 1 not shown.


Fig. 8. Candidate threshold values (CTV) plotted against $r^{2}$ for the applicable piecewise regression (data from Tables 10 and 11). Horizontal lines at 100 and $1000 \mathrm{ug} / \mathrm{L}$ are for reference. CTVs < 1 not shown.


Fig. 9. Candidate threshold values (CTV) from interpolation plotted against $r^{2}$ (data from Tables 1213). CTVs based on interpolation of median values at reference sites. Horizontal lines at 100 and $1000 \mathrm{ug} / \mathrm{L}$ are for reference. CTVs < 1 not shown.


Fig. 10. Candidate threshold values (CTV) from interpolation plotted against $r^{2}$ (data from Tables 1417). CTVs based on interpolation of median values at reference sites. Horizontal lines at 100 and $1000 \mathrm{ug} / \mathrm{L}$ are for reference. CTVs < 1 not shown.


Figure 11. Cumulative percentage distributions of nutrient concentration. Values on the $y$-axis are the percent of all sites with a value equal to or less than the corresponding $x$-axis value. Solid lines are for all Plains/Upper Midwest sites; dotted line is for all sites in Illinois and Indiana. Vertical lines correspond to reference lines in Figs. 7-10.


Figure 12. Nutrient candidate threshold values (CTV) for the Midwest. The Plains/Upper Midwest includes the following WSA aggregated ecoregions: Temperate Plains, Upper Midwest, Northern Plains, Southern Plains. EPA Nutrient Ecoregion (NER) VII is the "Corn Belt and Northern Great Plains." Illinois and Indiana are mostly in Robertson et al.'s N Zone 2 and mostly in P Zones 3 and 4. N criteria from Miltner (2010) are for DIN. Vertical reference lines correspond to lines in Fig. 11 and indicate the range of CTVs suggested by biotic responses (individual macroinvertebrate metrics) to nutrients. Filled symbols are from this report; open symbols are published values.

Appendix 1. Results of multiple linear models to predict metric values from natural factors and nutrients. Only factors with significant type III effects are shown. See Appendix 10 for variable definitions.


Appendix 2. Mean values for natural factors. See Appendix 10 for explanation of variables. WSA aggregate ecoregions: $\mathrm{SPL}=$ Southern Plains, $\mathrm{TPL}=$ Temperate Plains, UMW = Upper Midwest, NPL = Northern Plains. Other groups: CBP = Corn Belt Plains, IL = Illinois, IN = Indiana.

| Sites ( $n$ ) | Lat. <br> (dd) | Long. <br> (dd) | Mean width (m) | Mean Slope (\%) | Watershed Area $\left(\mathrm{km}^{2}\right)$ | Precipitation $(\mathrm{m} / \mathrm{y})$ | Substrate diameter (mm) | Wentworth name |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| All (327) | 42.82 | -69.56 | 8.02 | 0.72 | 1610 | 0.69 | 0.31 | Medium sand |
| TPL (130) | 42.39 | -93.17 | 8.12 | 0.59 | 948 | 0.81 | 0.35 | Medium sand |
| UMW (55) | 44.81 | -89.51 | 6.10 | 0.59 | 81 | 0.82 | 0.42 | Medium sand |
| NPL (94) | 45.32 | -103.24 | 7.50 | 0.92 | 2492 | 0.45 | 0.17 | Fine sand |
| SPL (48) | 36.82 | -100.72 | 10.94 | 0.83 | 3675 | 0.63 | 0.47 | Medium sand |
| CBP (61) | 41.89 | -92.24 | 8.64 | 0.52 | 251 | 0.84 | 0.38 | Medium sand |
| Not CBP (266) | 43.03 | -97.55 | 7.87 | 0.76 | 1940 | 0.65 | 0.29 | Medium sand |
| Reference (50) | 41.90 | -97.22 | 7.08 | 0.96 | 319 | 0.72 | 1.78 | Very coarse sand |
| Not Reference (277) | 42.98 | -96.44 | 8.18 | 0.67 | 1856 | 0.68 | 0.22 | Fine sand |
| IN and IL (28) | 40.23 | -92.24 | 7.01 | 1.01 | 123 | 0.97 | 0.46 | Medium sand |
| Not IN or IL (299) | 43.06 | -97.35 | 8.11 | 0.69 | 1757 | 0.66 | 0.30 | Medium sand |

Appendix 3. Significant ( $p<0.05$ ) spearman rank correlations among natural stream and stream location variables. Longitude values are negative in WSA data.

| Natural variable | Longitude <br> $(\mathrm{W})$ | Latitude <br> $(\mathrm{N})$ | Watershed <br> area | Precipitation | Substrate |
| :--- | ---: | ---: | ---: | ---: | ---: |
| Latitude $(\mathrm{N})$ | -0.23 |  |  |  |  |
| Watershed area $\left(\mathrm{km}^{2}\right)$ | -0.59 | 0.25 |  |  |  |
| Precipitation $(\mathrm{m} / \mathrm{y})$ | 0.82 | -0.52 | -0.64 | 0.26 | 0.20 |
| Mean log-transformed substrate diameter $(\mathrm{mm})$ | 0.12 | -0.22 |  | -0.20 | 0.16 |
| Mean slope $(\%)$ | -0.22 | 0.11 | 0.60 | 0.21 |  |
| Mean width $(\mathrm{m})$ |  |  | 0.6 |  |  |

Appendix 4. Mean diameter of substrate in streams dominated by coarse or fine substrates. WSA aggregate ecoregions: SPL = Southern Plains, TPL = Temperate Plains, UMW = Upper Midwest, NPL = Northern Plains. Other groups: CBP = Corn Belt Plains, IL = Illinois, IN = Indiana.

| Sites | Streams with coarse substrate (mean $\geq 1 \mathrm{~mm}$ ) |  |  |  | Streams with fine substrate (mean <1 mm) |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | \% of sites | $n$ | Mean diameter (mm) | Wentworth name | \% of sites | $n$ | Mean diameter (mm) | Wentworth name |
| All | 30 | 99 | 4.50 | Pebble | 70 | 228 | 0.10 | Very fine sand |
| TPL | 31 | 40 | 5.28 | Pebble | 69 | 90 | 0.10 | Very fine sand |
| UMW | 33 | 18 | 4.16 | Pebble | 67 | 37 | 0.13 | Fine sand |
| NPL | 21 | 20 | 3.33 | Granule | 79 | 74 | 0.08 | Very fine sand |
| SPL | 44 | 21 | 4.74 | Pebble | 56 | 27 | 0.08 | Very fine sand |
| CBP | 28 | 17 | 2.59 | Granule | 72 | 44 | 0.18 | Fine sand |
| Not CBP | 31 | 82 | 5.05 | Pebble | 69 | 184 | 0.08 | Very fine sand |
| Reference | 62 | 31 | 5.50 | Pebble | 38 | 19 | 0.28 | Medium sand |
| Not Reference | 25 | 68 | 4.17 | Pebble | 75 | 209 | 0.09 | Very fine sand |
| IN and IL | 39 | 11 | 6.82 | Pebble | 61 | 17 | 0.08 | Very fine sand |
| Not IN or IL | 29 | 88 | 4.27 | Pebble | 71 | 211 | 0.10 | Very fine sand |

Appendix 5. Summary of interpretations of biotic responses to nutrient concentration (plots in Appendix 4). $R t=$ response threshold; $R m=$ response midpoint; $S t=$ secondary threshold; CTV = Candidate threshold value; NA = not applicable. In every case, the default CTV is at Rt based on the hypothesized responses in Fig. 3.

| Plot | Metric | Nutrient | Plot type from Fig. 3 | Interpretation of CTV from plot |
| :---: | :---: | :---: | :---: | :---: |
| A1 | OLLEPTAX | Total N | F | Biotic response $R T$ at high nutrient value; highly tolerant organisms |
| A2 | OLLEPTAX | Total P | NA | Unreliable breakpoint at extreme of data |
| A3 | OLLEPTAX_residual error | Total N | F | Biotic response $R T$ at high nutrient value; highly tolerant organisms |
| A4 | OLLEPTAX_residual error | Total P | NA | Unreliable breakpoint at extreme of data |
| A5 | TL07RICH | Total N | C | Threshold set at midpoint of range of initial response. |
| A6 | TL07RICH | Total P | A | Apparent change in biotic response at $R t$; weak $S t$ |
| A7 | TL07RICH_residual error | Total N | A | Threshold set at $R t$; close match to hypothesized response |
| A8 | TL07RICH_residual error | Total P | A | Apparent change in biotic response at $R t$; weak $S t$ |
| A9 | HBI | Total N | B | Apparent change in biotic response at $R t$; weak $S t$ |
| A10 | HBI | Total P | B | Suspect break point at high value |
| A11 | HBI_residual error | Total N | B | Threshold set at $R t$; close match to hypothesized response |
| A12 | HBI_residual error | Total P | NA | Unreliable breakpoint at extreme of data |
| A13 | PLECRICH | Total N | A | Threshold set at $R m$ because of high variability at extreme of data |
| A14 | PLECRICH | Total P | G | Threshold set at midpoint of range of initial response. |
| A15 | PLECRICH_residual error | Total N | G | Threshold set at midpoint of range of initial response. |
| A16 | PLECRICH_residual error | Total P | A | Threshold set at $R t$; close match to hypothesized response |
| A17 | ODONRICH | Total N | A | Apparent change in biotic response at $R t$; weak $S t$ |
| A18 | ODONRICH | Total P | G | Threshold set at midpoint of range of initial response. |
| A19 | ODONRICH_residual error | Total N | A | Threshold set at $R t$; close match to hypothesized response |
| A20 | ODONRICH_residual error | Total P | A | Apparent change in biotic response at $R t$; weak $S t$ |
| A21 | CLMBRICH | Total N | E | Apparent change in biotic response at $R t$; close match to hypothesized response |
| A22 | CLMBRICH | Total P | E | Apparent change in biotic response at $R t$; close match to hypothesized response |
| A23 | CLMBRICH_residual error | Total N | NA | Unreliable breakpoint at extreme of data |
| A24 | CLMBRICH_residual error | Total P | A | Apparent change in biotic response at $R t$; weak $S t$ |
| A25 | TL89PTAX | Total N | H | Threshold set at midpoint of range of initial response. |
| A26 | TL89PTAX | Total P | NA | Unreliable breakpoint at extreme of data |
| A27 | TL89PTAX_residual error | Total N | H | Threshold set at $R m$ of range of initial response. |
| A28 | TL89PTAX_residual error | Total P | H | Threshold set at $R m$ mdpoint of range of initial response. |
| A29 | TL01RICH | Total N | A | Threshold set at $R m$ because of high variability at extreme of data |
| A30 | TL01RICH | Total P | A | Apparent change in biotic response at $R t$; weak $S t$ |
| A31 | TL01RICH_residual error | Total N | A | Threshold set at $R m$ because of high variability at extreme of data |
| A32 | TL01RICH_residual error | Total P | G | Threshold set at midpoint of range of initial response. |
| A33 | SHRDRICH | Total N | C | Threshold set at midpoint of range of initial response. |
| A34 | SHRDRICH | Total P | G | Threshold set at midpoint of range of initial response. |


| A35 | SHRDRICH_residual error | Total N | C | Threshold set at midpoint of range of initial response. |
| :---: | :---: | :---: | :---: | :---: |
| A36 | SHRDRICH_residual error | Total P | G | Threshold set at midpoint of range of initial response. |
| A37 | HABT_PT | Total N | A | Threshold set at $R m$ because of high variability at extreme of data |
| A38 | HABT_PT | Total P | C | Threshold set at midpoint of range of initial response. |
| A39 | HABT_PT_residual error | Total N | C | Threshold set at midpoint of range of initial response. |
| A40 | HABT_PT_residual error | Total P | C | Threshold set at midpoint of range of initial response. |
| A41 | TL67RICH | Total N | A | Apparent change in biotic response at $R t$; close match to hypothesized response |
| A42 | TL67RICH | Total P | A | Apparent change in biotic response at $R t$; close match to hypothesized response |
| A43 | TL67RICH_residual error | Total N | C | Threshold set at midpoint of range of initial response. |
| A44 | TL67RICH_residual error | Total P | A | Apparent change in biotic response at $R t$ |
| A45 | SCRPRICH | Total N | G | Threshold set at midpoint of range of initial response. |
| A46 | SCRPRICH | Total P | NA | Unreliable breakpoint at extreme of data |
| A47 | SCRPRICH_residual error | Total N | NA | No breakpoint |
| A48 | SCRPRICH_residual error | Total P | C | Threshold set at midpoint of range of initial response. |
| A49 | TOLR_PT | Total N | C | Threshold set at midpoint of range of initial response. |
| A50 | TOLR_PT | Total P | NA | Unreliable breakpoint at extreme of data |
| A51 | TOLR_PT_residual error | Total N | C | Threshold set at midpoint of range of initial response. |
| A52 | TOLR_PT_residual error | Total P | NA | Unreliable breakpoint at extreme of data |
| A53 | TL03PIND | Total N | A | Threshold set at $R m$ because of high variability at extreme of data |
| A54 | TL03PIND | Total P | A | Threshold set at $R m$ because of high variability at extreme of data |
| A55 | TL03PIND_residual error | Total N | A | Threshold set at $R m$ because of high variability at extreme of data |
| A56 | TL03PIND_residual error | Total P | NA | Unreliable breakpoint at extreme of data |
| A57 | MMI_WSABEST | Total N | C | Threshold set at midpoint of range of initial response. |
| A58 | MMI_WSABEST | Total P | NA | No breakpoint |
| A59 | MMI_WSABEST_residual error | Total N | NA | Unreliable breakpoint at extreme of data |
| A60 | MMI_WSABEST_residual error | Total P | NA | No breakpoint |
| A61 | CHIRRICH | Total N | C | Threshold set at midpoint of range of initial response. |
| A62 | CHIRRICH | Total P | E | Threshold set at $R t$; close match to hypothesized response |
| A63 | CHIRRICH_residual error | Total N | C | Threshold set at midpoint of range of initial response. |
| A64 | CHIRRICH_residual error | Total P | E | Threshold set at $R t$; close match to hypothesized response |
| A65 | PREDRICH | Total N | NA | Unreliable breakpoint at extreme of data |
| A66 | PREDRICH | Total P | E | Threshold set at $R t$; close match to hypothesized response |
| A67 | PREDRICH_residual error | Total N | E | Threshold set at St because of high variability at extreme of data |
| A68 | PREDRICH_residual error | Total P | G | Threshold set at midpoint of range of initial response. |
| A69 | HPRIME | Total N | C | Threshold set at midpoint of range of initial response. |
| A70 | HPRIME | Total P | E | Suspect breakpoint at high value (range of few data) |
| A71 | HPRIME_residual error | Total N | C | Threshold set at midpoint of range of initial response. |
| A72 | HPRIME_residual error | Total P | E | Suspect breakpoint at high value (range of few data) |
| A73 | SPRLRICH | Total N | C | Threshold set at midpoint of range of initial response. |


| A74 | SPRLRICH | Total P | E | Threshold set at $R t$; close match to hypothesized response |
| :---: | :---: | :---: | :---: | :---: |
| A75 | SPRLRICH_residual error | Total N | C | Threshold set at midpoint of range of initial response. |
| A76 | SPRLRICH_residual error | Total P | G | Threshold set at midpoint of range of initial response. |
| A77 | INTLRICH | Total N | A | Threshold set at Rm because of high variability at extreme of data |
| A78 | INTLRICH | Total P | G | Threshold set at midpoint of range of initial response. |
| A79 | INTLRICH_residual error | Total N | A | Threshold set at Rm because of high variability at extreme of data |
| A80 | INTLRICH_residual error | Total P | G | Threshold set at midpoint of range of initial response. |
| A81 | FEED_PT | Total N | A | Threshold set at Rm because of high variability at extreme of data |
| A82 | FEED_PT | Total P | A | Apparent change in biotic response at $R t$; weak $S t$ |
| A83 | FEED_PT_residual error | Total N | A | Apparent change in biotic response at $R t$; weak $S t$ |
| A84 | FEED_PT_residual error | Total P | A | Apparent change in biotic response at $R t$; weak $S t$ |
| A85 | COGARICH | Total N | C | Threshold set at midpoint of range of initial response. |
| A86 | COGARICH | Total P | E | Threshold set at $R t$; close match to hypothesized response |
| A87 | COGARICH_residual error | Total N | C | Threshold set at midpoint of range of initial response. |
| A88 | COGARICH_residual error | Total P | E | Threshold set at Rt; close match to hypothesized response |
| A89 | EPT_RICH | Total N | A | Threshold set at Rm because of high variability at extreme of data |
| A90 | EPT_RICH | Total P | A | Threshold set at Rm because of high variability at extreme of data |
| A91 | EPT_RICH_residual error | Total N | A | Threshold set at Rm because of high variability at extreme of data |
| A92 | EPT_RICH_residual error | Total P | G | Threshold set at midpoint of range of initial response. |
| A93 | EPHE_PT | Total N | A | Apparent change in biotic response at $R t$; weak $S t$ |
| A94 | EPHE_PT | Total P | G | Threshold set at midpoint of range of initial response. |
| A95 | EPHE_PT_residual error | Total N | NA | Unreliable breakpoint at extreme of data |
| A96 | EPHE_PT_residual error | Total P | NA | No breakpoint |

Appendix 6. Plots of relationships between nutrient concentration and raw macroinvertebrate metrics and between nutrient concentration and residual error from natural variation models. Vertical line is the CTV from Tables 9 and 10.

























Appendix 7. Plots of relationships between nutrient concentration and raw macroinvertebrate metrics for streams with fine substrate ( $<1 \mathrm{~mm}$ ) and streams with coarse substrate ( $\geq 1 \mathrm{~mm}$ ). Dashed vertical lines are CTVs for all streams for comparison. Solid vertical lines are CTVs from Tables 10 and 11; $r^{2}$ values show for each metric on plot; $r^{2}$ values between plots are for all streams for comparison. The rationale for identifying CTV is the same as described in Appendix 3.










Fine substrate








Log-transformed total P (ug/L)

Appendix 8. Plots of simple linear regressions with $50 \%$ prediction intervals between nutrient concentration and raw macroinvertebrate metrics. Horizontal lines are median values from reference sites. Details in Tables 12-13.






Appendix 9. Plots of simple linear regressions with between nutrient concentration and raw macroinvertebrate metrics for streams with fine and coarse substrate. Horizontal lines are median values from reference sites. Details in Tables 14-17.
















Appendix 10. Predictive and response variables used in this report.

| Variable | Explanation | Source |
| :---: | :---: | :---: |
| PREDRICH | Predator Distinct Taxa Richness | WSA |
| TL07RICH | PTV 0-7.9 Distinct Taxa Richness | WSA |
| TL01RICH | Percent tolerance value $=0-1.9$ Distinct Taxa Richness | WSA |
| EPHE_PT | Richness Metric Score for Nationwide MMI based on Ephemeroptera richness | WSA |
| ODONRICH | Odonata Distinct Taxa Richness | WSA |
| MMI_WSABEST | Best 6 WSA MMI | WSA |
| SPRLRICH | Sprawler Distinct Taxa Richness | WSA |
| INTLRICH | Intolerant Distinct Taxa Richness | WSA |
| CLMBRICH | Climber Distinct Taxa Richness | WSA |
| EPT_RICH | EPT Distinct Taxa Richness | WSA |
| PLECRICH | Plecoptera Distinct Taxa Richness | WSA |
| TL67RICH | PTV 6-7.9 Distinct Taxa Richness | WSA |
| FEED_PT | FFG Best 6 MMI Scoring | WSA |
| COGARICH | Collector-Gatherer Distinct Taxa Richness | WSA |
| HPRIME | Shannon Diversity | WSA |
| SHRDRICH | Shredder Distinct Taxa Richness | WSA |
| HABT_PT | Habit Best 6 MMI Scoring | WSA |
| CHIRRICH | Chironomid Distinct Taxa Richness | WSA |
| TL03PIND | PTV 0-3.9 \% Individuals | WSA |
| SCRPRICH | Scraper Distinct Taxa Richness | WSA |
| TOLR_PT | Tolerance Best 6 MMI Scoring | WSA |
| TL89PTAX | PTV 8-10 \% Distinct Taxa | WSA |
| HBI | Hilsenhoff Biotic Index | WSA |
| OLLEPTAX | Oligochaete/Leech \% Distinct Taxa | WSA |
| \% row crops | NLCD class 82 "cultivated crops" | NLCD 2001 |
| \% pasture/hay | NLCD class 81 "pasture/hay" | NLCD 2001 |
| \% developed | Sum of NLCD classes 21-24 "developed open space, low, medium and high intensity" | NLCD 2001 |
| \% forest | Sum of NLCD classes 41-43 "deciduous, evergreen and mixed forest" | NLCD 2001 |
| \% wetlands | Sum of NLCD classes 90 and 95 "woody wetlands, emergent herbaceous wetlands" | NLCD 2001 |
| Population density | 1990 Human population density (WSA variable name POPDENS) | WSA |
| Road density | Road density (WSA variable name RDDENS) | WSA |
| Riparian disturbance | Proximity weighted index based on presence of human disturbance (WSA variable name W1_HALL) | WSA |
| Riparian canopy openness | 100 - mean stream bank canopy density (WSA variable name XCDENBK) | WSA |
| Mean width | Mean channel width in m (WSA variable name XWIDTH) | WSA |
| Mean slope | Mean channel width in m (WSA variable name XSLOPE) | WSA |
| Watershed area | Watershed area (WS area) in km2 (WSA variable name LANDAREA) | WSA |
| Precipitation | Annual precipitation in m (WSA variable name PRECIP_M | WSA |
| Substrate | Log-transformed mean diameter of substrate (WSA variable name LSUB_DMM) | WSA |
| Latitude | WSA variable name LAT_DD | WSA |
| Longitude | WSA variable name LON_DD | WSA |

Appendix11. Tables $10-14$ with all parameters back transformed. CTVs for total N based on interpretation of piecewise regression models. $\mathrm{Mn}=\mathrm{minimum}$ observed nutrient concentration, $R t=$ response threshold, $S t=$ secondary threshold, $M x=$ maximum nutrient value, CTV = interpreted threshold value. See Fig. 3 and text for explanation of terms. $M n$, St, or Mx listed only if used to calculate Rm. Missing values for CTV are for relationships without reliable breakpoints. Metric values decrease with increasing nutrients unless indicated by " $(+)$ ". In some cases, CTV does not match exactly any of the parameters because of rounding errors in back-transformation of means.

| Metric | Piecewise model interpretation based on raw metrics |  |  |  |  |  |  | Piecewise model interpretation based on regression residuals |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $r^{2}$ | Mn | Rt | St or Mx | Rm | CTV | $\begin{gathered} \text { CTV } \\ (\mathrm{ug} / \mathrm{L}) \end{gathered}$ | $r^{2}$ | Mn | Rt | St or Mx | Rm | CTV | $\begin{gathered} \hline \text { CTV } \\ (\mathrm{ug} / \mathrm{L}) \end{gathered}$ |
| OLLEPTAX (+) | 0.15 |  | 12022 | 43651 | 22908 | 4.08 | 12022 | 0.12 |  | 12022 | 43651 | 22908 | 4.08 | 12022 |
| TL07RICH | 0.16 | 109 | 1287 |  | 375 | 2.58 | 379 | 0.18 |  | 330 | 740 | 494 | 2.52 | 330 |
| HBI (+) | 0.14 |  | 793 | 831 | 812 | 2.91 | 812 | 0.09 |  | 244 | 1022 | 500 | 2.39 | 244 |
| PLECRICH | 0.22 |  | 208 | 406 | 291 | 2.47 | 294 | 0.14 | 109 |  | 478 | 228 | 2.36 | 228 |
| ODONRICH | 0.09 |  | 512 | 645 | 574 | 2.71 | 512 | 0.10 |  | 388 | 43651 | 4120 | 2.59 | 388 |
| CLMBRICH | 0.08 |  | 330 | 43651 | 3801 | 2.52 | 330 | 0.12 |  | 2454 | 43651 | 10350 |  |  |
| TL89PTAX (+) | 0.08 | 109 |  | 1348 | 384 | 2.59 | 388 | 0.03 | 109 |  | 1379 | 388 | 2.59 | 388 |
| TL01RICH | 0.25 |  | 199 | 426 | 291 | 2.47 | 294 | 0.21 |  | 190 | 740 | 375 | 2.58 | 379 |
| SHRDRICH | 0.09 | 109 | 758 |  | 287 | 2.46 | 287 | 0.09 | 109 |  | 850 | 304 | 2.49 | 308 |
| HABT_PT | 0.07 |  | 274 | 274 | 274 | 2.44 | 274 | 0.04 | 109 |  | 2137 | 483 | 2.69 | 489 |
| TL67RICH | 0.06 |  | 315 | 1201 | 616 | 2.50 | 315 | 0.08 | 109 |  | 2041 | 472 | 2.68 | 478 |
| SCRPRICH | 0.04 | 109 | 1287 |  | 375 | 2.58 | 379 | 0.04 | 109 |  | 43651 | 2187 |  |  |
| TOLR_PT | 0.09 | 109 | 1121 |  | 350 | 2.55 | 354 | 0.05 | 109 |  | 1022 | 334 | 2.53 | 338 |
| TL03PIND | 0.11 |  | 208 | 1022 | 461 | 2.67 | 467 | 0.09 |  | 208 | 932 | 441 | 2.65 | 446 |
| MMI_WSABEST | 0.14 | 109 | 1379 |  | 388 | 2.59 | 388 | 0.07 |  | 147 | 157 | 152 |  |  |
| CHIRRICH | 0.07 | 109 | 4265 |  | 683 | 2.84 | 691 | 0.11 | 109 |  | 9771 | 1034 | 3.02 | 1046 |
| PREDRICH | 0.17 |  | 144 | 169 | 156 |  |  | 0.16 |  | 122 | 190 | 152 | 2.28 | 190 |
| HPRIME | 0.08 | 109 |  | 1904 | 456 | 2.66 | 456 | 0.14 | 109 |  | 1904 | 456 | 2.66 | 456 |
| SPRLRICH | 0.12 | 109 |  | 1174 | 358 | 2.56 | 362 | 0.16 | 109 |  | 976 | 326 | 2.52 | 330 |
| INTLRICH | 0.21 |  | 233 | 426 | 315 | 2.50 | 315 | 0.21 |  | 223 | 740 | 406 | 2.61 | 406 |
| FEED_PT | 0.07 |  | 338 | 416 | 375 | 2.58 | 379 | 0.04 |  | 3714 | 3889 | 3801 | 3.57 | 3714 |
| COGARICH | 0.09 | 109 |  | 1317 | 379 | 2.58 | 379 | 0.12 | 109 |  | 9999 | 1046 | 3.02 | 1046 |
| EPT_RICH | 0.15 |  | 233 | 406 | 308 | 2.49 | 308 | 0.13 |  | 228 | 561 | 358 | 2.56 | 362 |
| EPHE_PT | 0.12 |  | 1095 | 1201 | 1147 | 3.04 | 1095 | 0.05 | 109 |  | 43651 | 2187 |  |  |
| Median | 0.10 |  |  |  |  |  | 379 | 0.11 |  |  |  |  |  | 388 |

Appendix 11, continued. CTVs for total P based on interpretation of piecewise regression models. $M n=$ minimum observed nutrient concentration, $R t=$ response threshold, St $=$ secondary threshold, Mx = maximum nutrient value, CTV = interpreted threshold value. See Fig. 3 and text for explanation of terms. Mn, St, or Mx listed only if used to calculate Rm.

Missing values for CTV are for relationships without reliable breakpoints. Metric values decrease with increasing nutrients unless indicated by " $(+)$ ". In some cases, CTV does not match exactly any of the parameters because of rounding errors in back-transformation of means.

| Metric | Piecewise model interpretation based on raw metrics |  |  |  |  |  |  | Piecewise model interpretation based on regression residuals |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $r^{2}$ | Mn | Rt | $\begin{gathered} \text { St or } \\ M x \end{gathered}$ | Rm | CTV | $\begin{gathered} \text { CTV } \\ (\mathrm{ug} / \mathrm{L}) \end{gathered}$ | $r^{2}$ | Mn | Rt | $\begin{gathered} \text { St or } \\ M x \\ \hline \end{gathered}$ | Rm | CTV | $\begin{gathered} \text { CTV } \\ (\mathrm{ug} / \mathrm{L}) \end{gathered}$ |
| OLLEPTAX (+) | 0.05 | 2 |  | 5369 | 126 |  |  | 0.06 | 2 |  | 5369 | 126 |  |  |
| TL07RICH | 0.22 |  | 34 | 35 | 35 | 1.55 | 34 | 0.17 |  | 42 | 42 | 42 | 1.63 | 42 |
| HBI (+) | 0.09 |  | 561 | 1174 | 812 | 2.75 | 561 | 0.05 | 2 |  | 5369 | 126 |  |  |
| PLECRICH | 0.10 | 2 |  | 51 | 12 | 1.10 | 12 | 0.10 |  | 18 | 30 | 18 | 1.39 | 24 |
| ODONRICH | 0.05 | 2 |  | 101 | 17 | 1.25 | 17 | 0.06 |  | 59 | 59 | 59 | 1.78 | 59 |
| CLMBRICH | 0.09 |  | 31 | 5369 | 411 | 1.50 | 31 | 0.10 |  | 53 | 61 | 57 | 1.76 | 57 |
| TL89PTAX (+) | 0.08 | 2 |  | 5369 | 126 |  |  | 0.05 | 2 |  | 456 | 36 | 1.57 | 36 |
| TL01RICH | 0.18 |  | 21 | 21 | 21 | 1.34 | 21 | 0.16 | 2 |  | 104 | 17 | 1.25 | 17 |
| SHRDRICH | 0.11 | 2 |  | 94 | 16 | 1.23 | 16 | 0.09 | 2 |  | 99 | 16 | 1.24 | 16 |
| HABT_PT | 0.10 | 2 |  | 13 | 6 | 0.82 | 6 | 0.05 | 2 |  | 13 | 6 | 0.82 | 6 |
| TL67RICH | 0.11 |  | 29 | 308 | 96 | 1.48 | 29 | 0.09 |  | 34 | 61 | 45 | 1.54 | 34 |
| SCRPRICH | 0.10 | 2 |  | 5369 | 126 |  |  | 0.07 | 2 | 150 |  | 20 | 1.33 | 20 |
| TOLR_PT | 0.07 | 2 |  | 5369 | 126 |  |  | 0.05 |  | 5 | 7 | 6 | 0.9 | 7 |
| TL03PIND | 0.07 |  | 6 | 10 | 8 | 0.96 | 8 | 0.06 |  | 6 | 7 | 6 |  |  |
| MMI_WSABEST | 0.11 | 2 |  | 5369 | 126 |  |  | 0.06 | 2 |  | 5369 | 126 |  |  |
| CHIRRICH | 0.11 |  | 27 | 5369 | 388 | 1.45 | 27 | 0.07 |  | 27 | 5369 | 388 | 1.45 | 27 |
| PREDRICH | 0.11 |  | 18 | 5369 | 315 | 1.27 | 18 | 0.06 | 2 |  | 90 | 16 | 1.22 | 16 |
| HPRIME | 0.14 |  | 371 | 5369 | 1412 | 2.57 | 371 | 0.10 |  | 602 | 5369 | 1798 | 2.78 | 602 |
| SPRLRICH | 0.15 |  | 16 | 43651 | 850 | 1.22 | 16 | 0.12 | 2 |  | 94 | 16 | 1.23 | 16 |
| INTLRICH | 0.19 | 2 |  | 55 | 12 | 1.12 | 12 | 0.17 | 2 |  | 101 | 17 | 1.25 | 17 |
| FEED_PT | 0.08 |  | 40 | 41 | 40 | 1.61 | 40 | 0.06 |  | 40 | 41 | 40 | 1.61 | 40 |
| COGARICH | 0.16 |  | 15 | 5369 | 294 | 1.21 | 15 | 0.12 |  | 9 | 5369 | 231 | 1 | 9 |
| EPT_RICH | 0.13 |  | 9 | 12 | 10 | 1.05 | 10 | 0.09 | 2 |  | 86 | 15 | 1.21 | 15 |
| EPHE_PT | 0.04 | 2 |  | 48 | 11 | 1.09 | 11 | 0.02 | 2 |  | 5369 | 126 |  |  |
| Median | 0.11 |  |  |  |  |  | 17 | 0.07 |  |  |  |  |  | 20 |

Appendix 11, continued. CTVs for total N based on interpretation of piecewise regression models with data grouped by mean dominant substrate size. $M n=$ minimum observed nutrient concentration, $R t=$ response threshold, $S t=$ secondary threshold, $M x=$ maximum nutrient value. See Fig. 3 and text for explanation of terms. $M n$, St, or $M x$ listed only if used
to calculate $R m$. Missing values for CTV are relationships without reliable breakpoints. Metric values decrease with increasing nutrients unless indicated by " $(+)$ ".In some cases, CTV does not match exactly any of the parameters because of rounding errors in back-transformation of means.

| Metric | Piecewise model interpretation based on raw metrics for streams with fine substrates ( $<1 \mathrm{~mm}$ ) |  |  |  |  |  |  | Piecewise model interpretation base on raw metrics for streams with coarse substrates ( $\geq 1 \mathrm{~mm}$ ) |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $r^{2}$ | Mn | $R t$ | $S t$ or <br> Mx | Rm | CTV | $\begin{gathered} \text { CTV } \\ (\mathrm{ug} / \mathrm{L}) \\ \hline \end{gathered}$ | $r^{2}$ | Mn | $R t$ | St or Mx | Rm | CTV | $\begin{gathered} \text { CTV } \\ (\mathrm{ug} / \mathrm{L}) \\ \hline \end{gathered}$ |
| OLLEPTAX (+) | 0.13 |  | 12022 | 21379 | 16031 | 4.08 | 12022 | 0.15 | 114 |  |  | 114 |  |  |
| TL07RICH | 0.16 |  | 330 | 416 | 371 | 2.57 | 371 | 0.18 | 114 | 1697 |  | 441 | 2.65 | 446 |
| HBI (+) | 0.11 |  | 793 | 1022 | 901 | 2.96 | 911 | 0.14 |  | 911 | 43651 | 6309 | 2.96 | 911 |
| PLECRICH | 0.22 |  | 330 | 338 | 334 | 2.53 | 338 | 0.22 |  | 203 | 315 | 253 | 2.41 | 256 |
| ODONRICH | 0.12 |  | 536 | 549 | 542 | 2.74 | 549 | 0.12 |  | 111 | 43651 | 2212 |  |  |
| CLMBRICH | 0.09 |  | 561 | 561 | 561 | 2.75 | 561 | 0.11 |  | 2817 | 43651 | 11091 | 3.45 | 2817 |
| TL89PTAX (+) | 0.11 |  | 793 | 1022 | 901 | 2.96 | 911 | 0.07 | 114 | 932 |  | 326 | 2.52 | 330 |
| TL01RICH | 0.20 | 109 | 758 |  | 287 | 2.46 | 287 | 0.27 |  | 203 | 301 | 247 | 2.40 | 250 |
| SHRDRICH | 0.07 |  | 346 | 362 | 354 | 2.55 | 354 | 0.21 |  | 173 | 1229 | 461 | 2.67 | 467 |
| HABT_PT | 0.05 |  | 1022 | 1046 | 1034 | 3.02 | 1046 | 0.18 | 114 | 1659 |  | 436 | 2.64 | 436 |
| TL67RICH | 0.08 |  | 346 | 536 | 431 | 2.64 | 436 | 0.09 |  | 588 | 1022 | 775 | 2.77 | 588 |
| SCRPRICH | 0.02 |  | 233 | 21379 | 2238 |  |  | 0.07 | 114 | 2817 |  | 568 | 2.76 | 574 |
| TOLR_PT | 0.06 | 109 | 954 |  | 323 | 2.51 | 323 | 0.10 | 114 | 723 |  | 287 | 2.46 | 287 |
| TL03PIND | 0.08 |  | 190 | 1317 | 500 | 2.70 | 500 | 0.17 | 114 | 1697 |  | 441 | 2.65 | 446 |
| MMI_WSABEST | 0.11 | 109 | 2041 |  | 472 | 2.68 | 478 | 0.13 |  | 208 | 489 | 319 | 2.51 | 323 |
| CHIRRICH | 0.09 | 109 | 1022 |  | 334 | 2.53 | 338 | 0.06 | 114 | 8510 |  | 988 |  |  |
| PREDRICH | 0.12 | 109 | 561 |  | 247 |  |  | 0.07 | 114 |  |  | 114 |  |  |
| HPRIME | 0.06 | 109 | 1348 |  | 384 | 2.59 | 388 | 0.07 |  | 388 | 1317 | 715 | 2.59 | 388 |
| SPRLRICH | 0.09 | 109 | 911 |  | 315 | 2.50 | 315 | 0.12 |  | 194 | 1046 | 451 | 2.66 | 456 |
| INTLRICH | 0.16 |  | 379 | 379 | 379 | 2.58 | 379 | 0.19 | 114 | 1659 |  | 436 | 2.64 | 436 |
| FEED_PT | 0.06 |  | 244 | 561 | 371 | 2.57 | 371 | 0.08 | 114 | 131 |  | 122 |  |  |
| COGARICH | 0.10 | 109 | 999 |  | 330 | 2.52 | 330 | 0.07 |  | 388 | 1348 | 723 | 2.59 | 388 |
| EPT_RICH | 0.11 |  | 268 | 426 | 338 | 2.53 | 338 | 0.13 | 114 | 1904 |  | 467 | 2.67 | 467 |
| EPHE_PT | 0.10 |  | 793 | 1994 | 1258 | 3.10 | 1258 | 0.09 | 114 | 128 |  | 121 |  |  |
| Median | 0.10 |  |  |  |  |  | 384 | 0.12 |  |  |  |  |  | 441 |

Appendix 11, continued. CTVs for total P based on interpretation of piecewise regression models with data grouped by mean dominant substrate size. $M n=$ minimum observed nutrient concentration, $R t=$ response threshold, $S t=$ secondary threshold, $M x=$ maximum nutrient value. See Fig. 3 and text for explanation of terms. $M n$, $S t$, or $M x$ listed only if used
to calculate $R m$. Missing values for CTV are for relationships without reliable breakpoints. Metric values decrease with increasing nutrients unless indicated by "( + )".In some cases, CTV does not match exactly any of the parameters because of rounding errors in back-transformation of means.

| Metric | Piecewise model interpretation based on raw metrics for streams with fine substrates ( $<1 \mathrm{~mm}$ ) |  |  |  |  |  |  | Piecewise model interpretation base on raw metrics for streams with coarse substrates ( $>1 \mathrm{~mm}$ ) |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $r^{2}$ | Mn | $R t$ | St or Mx | Rm | CTV | $\begin{gathered} \text { CTV } \\ (\mathrm{ug} / \mathrm{L}) \\ \hline \end{gathered}$ | $r^{2}$ | Mn | $R t$ | St or Mx | Rm | CTV | $\begin{gathered} \text { CTV } \\ (\mathrm{ug} / \mathrm{L}) \end{gathered}$ |
| OLLEPTAX (+) | 0.13 | 2 | 4073 |  | 110 |  |  | 0.16 |  | 71 | 549 | 199 | 1.86 | 71 |
| TL07RICH | 0.15 |  | 32 | 46 | 38 | 1.60 | 39 | 0.35 |  | 31 | 549 | 132 | 1.51 | 31 |
| HBI (+) | 0.07 |  | 561 | 1147 | 803 | 2.75 | 561 | 0.17 | 3 | 140 |  | 23 | 1.38 | 23 |
| PLECRICH | 0.07 | 2 | 25 |  | 8 | 0.95 | 8 | 0.31 |  | 19 | 19 | 19 | 1.31 | 19 |
| ODONRICH | 0.08 | 2 | 426 |  | 35 | 1.56 | 35 | 0.06 | 3 | 101 |  | 19 | 1.31 | 19 |
| CLMBRICH | 0.10 |  | 20 | 5369 | 334 | 1.32 | 20 | 0.09 | 3 | 6 |  | 4 |  |  |
| TL89PTAX (+) | 0.07 |  | 11 | 2238 | 163 |  |  | 0.15 | 3 | 190 |  | 27 | 1.45 | 27 |
| TL01RICH | 0.20 |  | 8 | 9 | 8 | 0.97 | 8 | 0.20 |  | 10 | 549 | 78 | 1.06 | 10 |
| SHRDRICH | 0.08 | 2 | 80 |  | 15 | 1.20 | 15 | 0.20 | 3 | 233 |  | 30 | 1.49 | 30 |
| HABT_PT | 0.06 | 2 | 1548 |  | 67 |  |  | 0.15 | 3 | 3 |  | 3 |  |  |
| TL67RICH | 0.11 |  | 46 | 51 | 49 | 1.70 | 49 | 0.25 |  | 20 | 25 | 23 | 1.42 | 25 |
| SCRPRICH | 0.07 |  | 19 | 5369 | 330 | 1.31 | 19 | 0.13 |  | 6 | 7 | 7 |  |  |
| TOLR_PT | 0.04 |  | 15 | 5369 | 294 | 1.21 | 15 | 0.17 |  | 323 | 549 | 421 |  |  |
| TL03PIND | 0.03 |  | 561 | 5369 | 1737 | 2.75 | 561 | 0.11 |  | 10 | 549 | 76 | 1.03 | 10 |
| MMI_WSABEST | 0.07 |  | 203 | 5369 | 1046 | 2.31 | 203 | 0.23 | 3 | 181 |  | 26 | 1.44 | 27 |
| CHIRRICH | 0.11 |  | 10 | 12 | 11 |  |  | 0.34 |  | 57 | 549 | 177 | 1.76 | 57 |
| PREDRICH | 0.06 |  | 758 | 5369 | 2017 |  |  | 0.23 |  | 10 | 549 | 77 | 1.04 | 10 |
| HPRIME | 0.12 |  | 388 | 5369 | 1444 | 2.59 | 388 | 0.19 |  | 31 | 549 | 132 | 1.51 | 31 |
| SPRLRICH | 0.12 |  | 10 | 12 | 11 |  |  | 0.35 |  | 35 | 549 | 140 | 1.56 | 35 |
| INTLRICH | 0.16 |  | 8 | 8 | 8 |  |  | 0.29 |  | 9 | 549 | 73 | 1.00 | 9 |
| FEED_PT | 0.06 | 2 |  | 5369 | 126 |  |  | 0.10 | 3 | 181 |  | 26 | 1.44 | 27 |
| COGARICH | 0.14 |  | 15 | 5369 | 294 | 1.21 | 15 | 0.25 |  | 35 | 549 | 140 | 1.56 | 35 |
| EPT_RICH | 0.09 |  | 9 | 9 | 9 |  |  | 0.22 |  | 10 | 45 | 22 | 1.36 | 22 |
| EPHE_PT | 0.02 | 2 |  | 5369 | 24 |  |  | 0.06 | 3 |  | 549 | 46 |  |  |
| Median | 0.08 |  |  |  |  |  | 28 | 0.20 |  |  |  |  |  | 27 |

Appendix 12: Curve for back transforming nutrient concentrations.


